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Also the Official Organ of the American Society for Experimental Pathology

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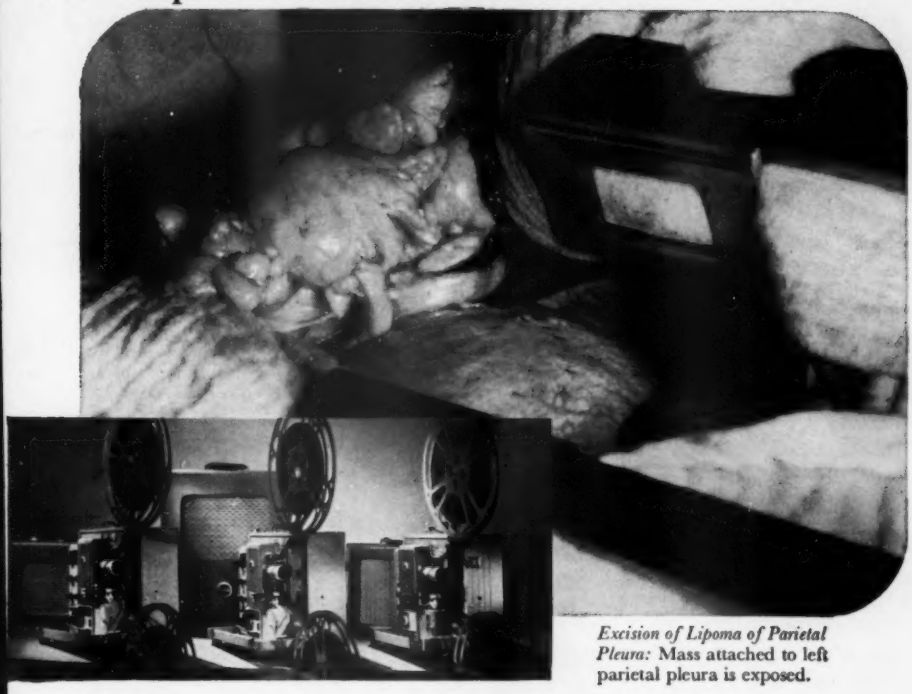

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A.M.A. ARCHIVES OF
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Effects of Altitude on Dogs with Valvular Heart Disease

Tolerance and Pathologic Effects of Acute and Chronic Exposures

PAUL D. ALTLAND, Ph.D.; BENJAMIN HIGHMAN, M.D., Bethesda, Md., and JOSEPH ROSHE, M.D., Indianapolis

The ability of patients with cardiovascular lesions to withstand exposure to altitude is uncertain and not well understood. It has been reported [1] that patients with a variety of cardiovascular diseases successfully withstood long journeys in planes with cabin pressure usually adjusted not to surpass 8,000 ft. equivalent. In another study,[2] however, cardiovascular disease of marked degree was noted in about three-fourths of those taken ill during flight. Patients with angina pectoris showed poor tolerance to exposures to 18,000 ft. simulated altitude.[3] Variable responses have been reported in animal experiments. Unanesthetized dogs with large three-day myocardial infarcts produced by coronary-artery ligation showed little, if any, reduction in tolerance when exposed to 34,000 ft. simulated altitude,[4] whereas anesthetized dogs [5] exposed soon after coronary-artery ligation failed to survive a short exposure to 8% oxygen (25,000 ft. equivalent). Further studies of the tolerance of animals with cardiovascular lesions are needed. This present study was designed to determine the effects of acute and chronic exposure to simulated high altitude on dogs with aortic and mitral insufficiency.

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National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare, Bethesda, Md., and Indiana University Medical Center.

Methods

Mongrel dogs of both sexes, weighing 14.5 to 21.4 kg., were selected. The ages of the dogs were unknown but were estimated, on the basis of the general physical condition, and particularly of the appearance of the incisor teeth, to range from 1 to 7 years. Aortic insufficiency was produced in 19 dogs by removing a disk of tissue from the base of the right coronary cusp [6] with a punch 3.9 or 4.9 mm. in diameter. In seven dogs, mitral insufficiency was produced by the transventricular severance of chordae tendineae [7]; mild to moderately severe degrees of aortic insufficiency were produced in four of the dogs with mitral insufficiency at a second operation. All dogs operated on were rested at least 30 days before use in altitude tests. Hemodynamic studies were made under pentobarbital anesthesia before and occasionally after exposures to high altitude. Retrograde left-heart catheterizations were performed using No. 7 Cournand catheters; pressures were recorded with a P23D Statham transducer connected to a direct recorder. The zero level was set at 7 cm. anterior to the skin of the back. Electrocardiograms were recorded on Sanborn Viso Cardiettes on unanesthetized and anesthetized dogs.

Exposure to Simulated High Altitude.—The tests were conducted in a large, adequately ventilated decompression chamber maintained at 23 to 25 C. In the chamber the dogs were unrestrained and had access to drinking water. Acute altitude tolerance tests were given normal dogs and dogs

operated on, in the following manner: The rate of climb was 2,000 ft/min. with five-minute rest periods at 10, 15, 20, and 25,000 ft.; all dogs were maintained at 30,000 ft. for four hours; after a 10-day interval, survivors were then exposed in the same manner at 32,000 ft.; after similar intervals they were then exposed to 34,000 ft. and, finally, to 36,000 ft. Autopsies were made only on nonsurvivors. The chronic altitude tests were conducted by exposing normal and surgically treated dogs approximately six hours a day, four to five days a week to 30,000 ft. from 11 to 96 times in a maximum of 164 days. Hematologic studies were conducted throughout this period. Dogs were killed at intervals for pathologic studies. The brain was fixed in Orth's solution and other tissues in a buffered (pH 7.0) 10% aqueous solution of formalin. Routine paraffin sections were stained with hematoxylin-azure-eosin and, in selected instances, with a variety of other stains. The method of Reinhardt and Abul-Haj was used to demonstrate acid mucopolysaccharides. Frozen sections of selected tissues were stained for fat with oil red O.[8]

Results

Acute Altitude Tolerance.—Two groups of dogs with aortic insufficiency (AI-1, AI-2) and two groups of dogs not operated on were selected for study. The degrees of aortic incompetence are shown in Table 1. Particularly noteworthy are the wide femoral pulse pressures and the marked increase in the left ventricular end diastolic pressure. The electrocardiograms from the dogs operated on (obtained while lying on their backs) showed significantly increased voltage of the P-wave in Lead III and V-A and a significant decrease in the voltage of the Q-wave in Leads I, II, and III when compared with preoperative values. The electrocardiograms showed no significant change after the altitude exposure. During the exposures the dogs usually lay quietly, only occasionally rising and displaying a staggering gait. All showed an initial hyperpnea for 10 to 30 minutes, followed by slow, deep breathing during the remainder of the exposure. There were no deaths at 30,000 and 32,000 ft. One of the 10 AI dogs died at 34,000 and 2 at 36,000 ft. One of the six controls died at 36,000 ft.

The AI dog which died at 34,000 ft. maintained a respiratory rate of about 84/min. for three hours, but five minutes before death breathing grew more and more shallow, until it stopped. The femoral artery pressure prior to exposure was 240/115 mm. Hg; the systolic pressure was the highest noted in any of the dogs. Autopsy revealed a few small pulmonary hemorrhages in the right lower lobe. Thickening of the anterior mitral leaflet above the insertions of the chordae and thickening of the rim of the defect in the right aortic leaflet were noted, but these changes can be attributed to the aortic insufficiency.

TABLE 1.—*Average Pressures of Dogs with Aortic and Mitral Insufficiency* *

		Femoral						
		Before Surgery			After Surgery			Left Ventricle End-Diast. (L.V.E.P.) Mm. Hg
		Mm. Hg		Pulse Pressure, Mm. Hg	Mm. Hg		Pulse Pressure, Mm. Hg	
Group	No. Dogs	Sys.	Diast.		Sys.	Diast.		
AI-1	5	--	--		193	82	111±6.6 †	--
Control	3	162	95	66±4.0	--	--	--	--
AI-2	5	155	97	59±4.0	198	72	126±1.8	17.1±3.0
Control	3	185	110	75±8.3	--	--	--	8.3±1.1
AI-3	5	153	94	59±5.0	209	85	124±15.1	16.8±2.4
Control	5	179	106	73±11.4	--	--	--	9.4±0.7
MI-1	5	--		--	176	100	76±8.3	13.2±0.7
Control	5	192	102	90±15.3	--	--	--	7.9±0.6
AI-MI-1	4	--		--	207	82	125±18.6	23.3±10.3
Control	4	--		--	--	--	--	--

* AI indicates aortic insufficiency; MI, mitral insufficiency.
† SEM

The second AI dog died after two hours at 36,000 ft. The femoral artery pressure was 200/75 prior to exposure. This dog showed small pulmonary hemorrhages in the left middle lobe, but no other significant changes. Another AI dog died after three hours at 36,000 ft. The preexposure femoral artery pressure was 200/70 mm. Hg. This dog showed a cerebral hemorrhage at the occipital pole, subendocardial hemorrhages in the left ventricle, and hemorrhages in the adrenal gland and mucosa of the bowel. The only fatality among the controls in the entire study was a dog which stopped breathing after three hours at 36,000 ft. He was promptly revived by recompression to ground level and administration of artificial respiration. He developed neurological symptoms and was killed preterminally 24 hours later. The preexposure femoral artery pressure was 160/90 mm. Hg. He showed a large cerebral hemorrhage in the left occipital lobe, a small hemorrhagic splenic infarct, a small hemorrhagic extravasation in the anterior mitral leaflet, and lobular pneumonia, with organizing venous thrombi in several veins.

One group, of five dogs, with mitral insufficiency (MI-1) and five controls not operated on were also subjected to the acute altitude tolerance test (Table 1). One dog with mitral insufficiency died after three hours at 36,000 ft.; he had a femoral artery pressure of 178/107 mm. Hg and a left ventricular end-diastolic pressure (L.V.E.P.) of 14.2 mm. Hg. At autopsy, the severance of the chordae tendineae to the posterior papillary muscle was confirmed, and the mitral leaflets were thickened and deformed. There was no evidence of acute heart failure. All controls survived.

Four dogs with combined aortic and mitral insufficiency (Table 1) and four controls not operated on were tested. Again, all controls survived, and only one of the four dogs with valvular insufficiency died, after two hours at 36,000 ft. His preexposure femoral arterial pressure was 220/110, with a mean of 140 mm. Hg. The left ventricular end diastolic pressure was 11 mm. Hg. Pathologic study revealed a small organizing sterile vegetation on the commissure between the left and the right aortic cusp, a small infarct in the spleen, and moderately extensive lobular pneumonia with scattered hemorrhages in the left lung and in the brain. One of these surviving dogs with severe mitral and aortic insufficiency had a left ventricular end-diastolic pressure of 42 mm. Hg; this emphasizes the high hypoxic resistance of dogs even with severe valvular insufficiency.

One dog with aortic insufficiency, used in therapeutic studies on experimental staphylococcal endocarditis[9] for three months before the altitude exposure, died after only 15 minutes at 30,000 ft. Pathologic studies revealed multiple small valvular vegetations, patchy myocarditis, and several old renal infarcts.

The lungs of the five dogs that died in the acute altitude tolerance test were stained for fat; two showed sudanophilic material (Fig. 1) in a few scattered alveolar vessels (fat emboli).

Chronic Altitude Tolerance.—Seventeen dogs not operated on and five dogs with aortic insufficiency (AI-3, Table 1) were exposed repeatedly to 30,000 ft. altitudes. Twelve of the normal dogs were killed after 11 to 37 exposures. The remainder, including the dogs operated on, were exposed until death, or a maximum of 96 exposures in 164 days. Two AI dogs died after 30 to 83 exposures, and three controls died after 30, 49, and 83 exposures, respectively. At the onset of the experiment the mean hematocrit values of the five control dogs chosen for prolonged exposures and the five AI dogs were 51% and 48%, respectively; after six weeks' exposure the values had increased to 70% and 71% and remained at these high levels throughout the experiment.

Pathology.—Visceral engorgement, increased cellularity of the bone marrow, cardiovascular lesions, and renal hemosiderosis were the most constant findings. The severity of the lesions varied markedly in

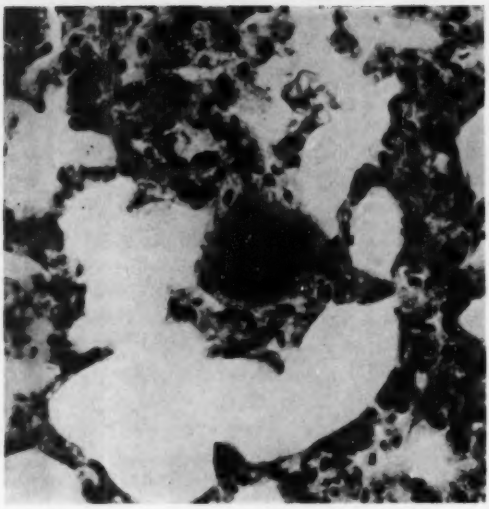


Fig. 1.—Lung of dog with combined mitral and aortic insufficiency (MI-AI) that died at 36,000 ft., showing dark sudanophilic material in alveolar vessel. Hematoxylin and oil red O; × 300.

different dogs, but there were no striking differences in the character and frequency of the lesions attributable to high altitude between dogs not operated on and dogs with aortic insufficiency.

Cardiovascular Lesions.—The cardiac valves, particularly the mitral and aortic, often showed thickening, with a large amount of metachromatic material and material staining like acid mucopolysaccharide. Patchy endocardial thickening was frequent, particularly along the mural surface of the papillary muscles of the left ventricle and, in dogs with aortic insufficiency, on the ventricular endocardium below the defect in the aortic leaflet. In one dog not operated on and four AI dogs exposed 83 to 96 times, several white, cord-like bands (Fig. 2) or broader plaques of thickened endocardium, 4 cm. in maximum diameter, were seen in the left ventricle on, along the border of, or between the papillary muscles. One control dog (Dog 10, Table 2) and one dog with aortic insufficiency (Dog 18), each dying after 30 exposures, showed a small sterile organizing vegetation on the left ventricular endocardium below the posterior mitral and right aortic leaflets, respectively (Figs. 3, 4). Several other dogs showed nodular thickenings of the valves or adjacent endocardium, suggesting healed small vegetations.

Heart-body weight ratio increased somewhat with prolonged exposure and was relatively greater in AI dogs. Several dogs showed foci of hemorrhage, subacute inflammation, or necrosis in the myocardium,

Fig. 2.—Heart of AI Dog 21 (Table 2) exposed 96 times to 30,000 ft. The aorta has been opened by bisecting the thickened anterior mitral leaflet, revealing the rounded defect at the base of the thickened right aortic cusp in the right upper portion of the Figure. On the left are several gray-white plaques and an elongated band of thickened endocardium on the lower anterior border of the anterior papillary muscle.

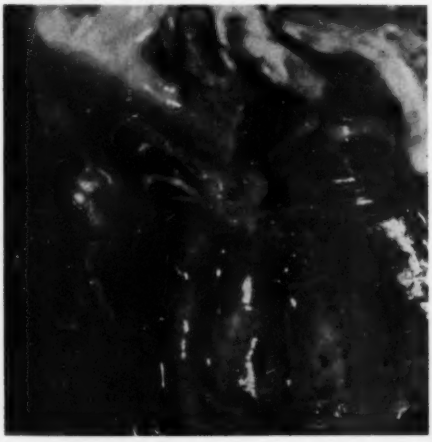


Fig. 3.—Section through right aortic leaflet of AI Dog 18, which died after 30 exposures to 30,000 ft. On the lower right are seen two sterile vegetations, one attached to the upper rim of the surgical defect (arrow), and the other, to the thickened ventricular endocardium immediately below the leaflet (arrow). On the left is the aorta, showing an arteriosclerotic plaque. Hematoxylin and eosin; × 6.

TABLE 2.—*Effects of Repeated Six-Hour Exposures to 30,000 Feet on Normal Dogs and on Dogs with Aortic Insufficiency*

Dog * No.	No. of Exposures	Aortic Plaques †	Infarcts ‡	Renal Hemosiderosis §
		Normal Dogs		
1	11	+	—	—
2	11	—	—	—
3	13	—	—	++
4	18	—	—	—
5	18	—	—	—
6	19	+	—	+
7	24	—	—	+
8	29	+++	—	—
9	29	++	—	+
10	30	—	S	—
11	34	—	—	+
12	35	++	—	—
13	37	—	—	—
14	49	+	—	+++
15	83	0	K	+
16	96	++	K	+++
17	96	++++	K	+
		Dogs with Aortic Insufficiency		
18	30	+++	K	—
19	83	—	K	+
20	83	+++	K	+++
21	96	+	K	++
22	96	++	—	++

* Dogs 9 to 13 died; all others were killed for study.

† Plaques are graded by their maximum thickness: +=less than 0.1 mm. and ++++, over 1 mm.; —=no plaques; 0, no microscopic study.

‡ S=spleen, and K, kidney.

§ +=slight; ++, moderate; +++, abundant hemosiderin deposits in kidney.

and one (Dog 20), killed preterminally after 83 exposures, showed a severe subacute myocarditis involving most of the right auricle and ventricle. After prolonged exposures, the dogs showed fibrous thickening of the tips of the left ventricular

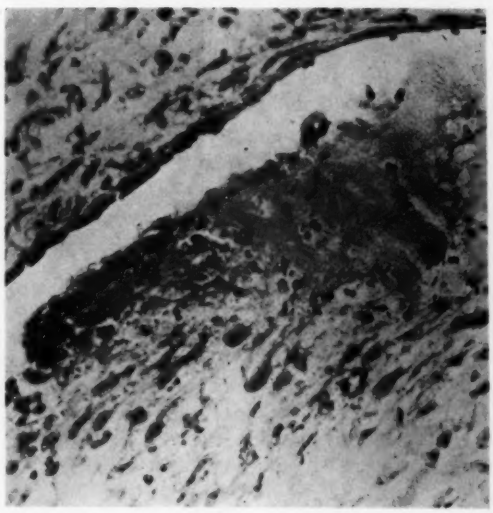


Fig. 4.—Higher magnification of portion of ventricular vegetation seen in Figure 3, showing partial covering by endothelium and fibroblastic organization of base of vegetation. Above is portion of right aortic leaflet. Hematoxylin and eosin; × 130.

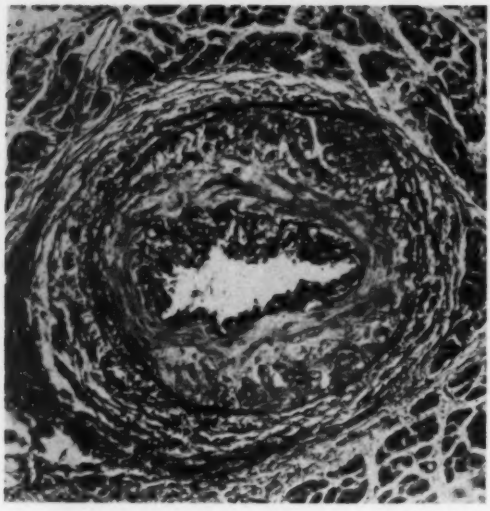


Fig. 5.—Section of artery in left auricle of AI Dog 21, killed after 96 exposures to 30,000 ft., showing thickening of adventitia and cellular proliferation of intima. Orcein elastica-Van Gieson stain; × 150.

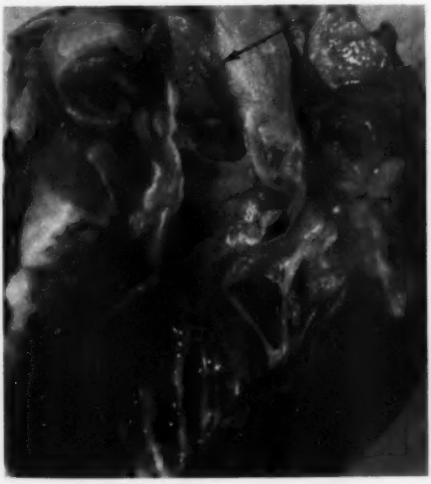
papillary muscles and, frequently, patchy interstitial fibrosis of the myocardium, particularly near the base of the heart and in the papillary muscles. However, similar changes were seen occasionally in unexposed dogs, chiefly those with mitral insufficiency.

The myocardial arteries of the exposed dogs often showed thickening of the adventitia and occasionally cellular proliferation in the intima (Fig. 5). Vascular changes were especially severe in the AI dog (middle-aged) that died after 30 exposures (Dog 18). Some arteries showed hemorrhages in the adventitia and outer media, with fibrinous exudate in the media, and some showed intimal hyaline changes and hyalinization or necrosis of the media.

Changes in the Aorta and Pulmonary Artery.—The ascending aorta was sectioned through each of the semilunar cusps and grossly appeared normal or showed slight roughening of the intimal surface with linear markings and occasionally grayish plaques and longitudinal ridges (Fig. 6). The remainder of the aorta was studied only in surgically untreated dogs that received not more than 37 exposures. In several dogs, there were apparent intimal thickenings at the ostia of arterial branches and, particularly near the arch and in the lower abdominal aorta, grayish-white small plaques and one or more longitudinal ridges, ranging from less than 0.5 to over 5 cm. in length and up to 2 mm. in width.

The ascending aortas of 11 of 16 dogs given 19 or more exposures (aorta of Dog 15, Table 2, not studied microscopically) showed intimal thickenings or

Fig. 6.—Heart of surgically untreated Dog 17, killed after 96 exposures. The aorta has been opened by bisecting the thickened anterior mitral valve and shows several elongated plaques above the right aortic cusp and the coronary ostium in the upper part of the figure.



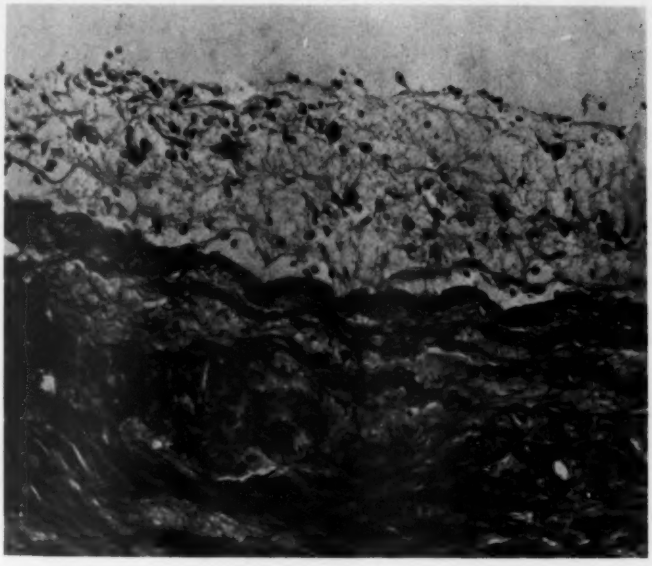


Fig. 7.—Section of arteriosclerotic plaque opposite right aortic leaflet in young surgically untreated Dog 6, killed after 19 exposures to 30,000 ft. The plaque has a loose cellular structure; the intercellular matrix stained intensely like acid mucopolysaccharide with the method of Reinhardt and Abul-Haj. The underlying superficial media shows some mucoid degeneration. Hematoxylin and eosin; × 200.

plaques, located most frequently at or slightly above the sinuses of Valsalva and near the ostia of the coronary vessels (Figs. 3 and 7). Similar plaques were seen occasionally in the coronary arteries (Fig. 8) near the ostia and in the pulmonary artery. The lesions in those that received less than 30 exposures were relatively thin and loosely cellular with a pale fibrillar intercellular matrix containing material staining like acid mucopolysaccharides (Fig. 7). The cells consisted chiefly of fixed tissue cells of the mesenchymal type admixed with large mononuclear, and occasionally binucleated, cells and inflammatory cells, chiefly lymphocytes and occasionally neutrophils. No lipophages were seen in the intima, but slight fatty, mucoid, and other degenerative changes were seen in the underlying media. The lesions in those that received more than 30 exposures were often much thicker; cells were fusiform and predominantly of the fibrocyte type, and the matrix showed abundant dense collagen but little or no material staining like acid mucopolysaccharide (Fig. 9). Some densely collagenized older plaques contained numerous, irregularly disposed elastic fibers and were surmounted by a loosely cellular layer. The media of some of these aortas showed foci of fibrosis and disruption of elastic fibers (Fig. 9). The thoracic and abdominal aorta showed similar arteriosclerotic lesions, but fibrosis tended to develop after fewer exposures.

Fig. 8.—Coronary artery in same section as Figure 7, showing pale, broad arteriosclerotic plaque, similar to that of the aorta (arrow). Hematoxylin and eosin; × 30.

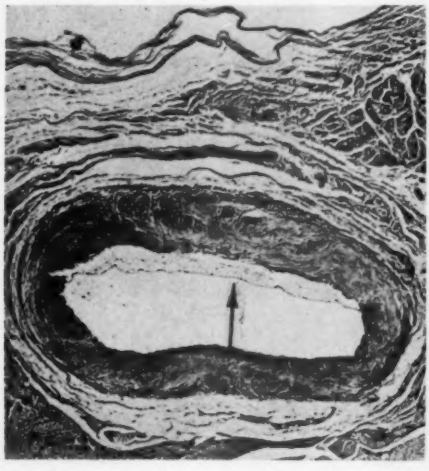


Fig. 9.—Section of aorta above right coronary cusp in surgically untreated Dog 17, killed after 96 exposures to 30,000 ft., showing thick, densely collagenized arteriosclerotic plaque. Orcein elastica-Van Gieson stain; ×30.

Changes in Other Organs.—Seven dogs had infarcts. A splenic infarct and a massive recent renal infarct were seen in Dogs 10 and 18 (one control and one AI), respectively. Both dogs died after 30 exposures, and each showed cardiac vegetations; in addition, the control had organizing venous thrombi in the right middle lobe. Old renal infarcts, but no demonstrable vegetations, were seen in five of the seven dogs that received 83 or more exposures (Table 2).

Hemosiderin deposits were seen in the epithelium of the renal convoluted tubules in all 8 dogs receiving 49 or more exposures and in 5 of 14 receiving 11 to 37 exposures. Necrosis of renal convoluted

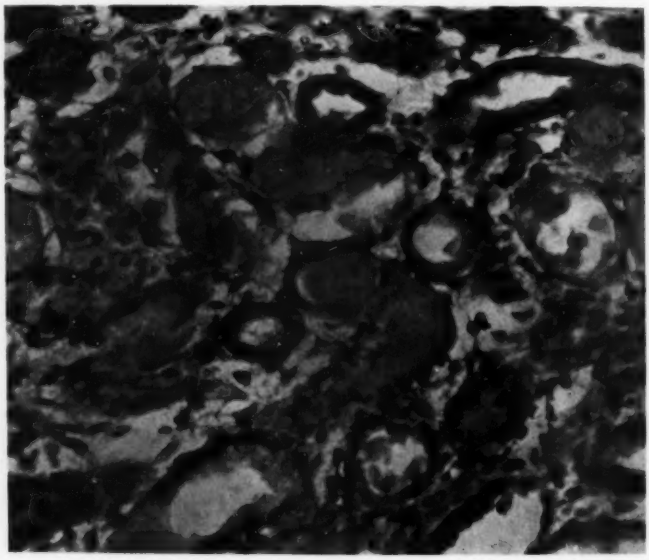
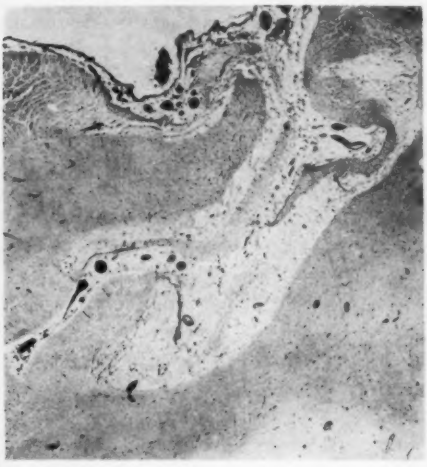


Fig. 10.—Necrosis of renal cortical tubules in surgically untreated Dog 4, killed after 18 exposures to 30,000 ft. Hematoxylin-azure-eosin stain; ×200.

Fig. 11.—Base of femoral epiphysis of surgically untreated Dog 17, killed after 96 exposures to 30,000 ft., showing network of dense fibrous trabeculae in hypercellular bone marrow. Van Gieson stain; × 200.

tubules (Fig. 10), less severe than that found 24 to 48 hours after an acute exposure to 38,000 ft.,[4] was seen in six dogs exposed 18 to 96 times. Interstitial hemorrhages were seen in the renal medulla of a control dog exposed 29 times and in an AI dog exposed 96 times.

Fig. 12.—Brain of surgically untreated, control Dog 17, killed after 96 exposures to 30,000 ft., showing large area of softening in gray matter of parietal lobe. Azure-eosin stain; × 6.



Marked pulmonary congestion with scattered hemorrhages was seen in the dogs that succumbed to the exposures. One dog not operated on that died after 49 exposures and one AI dog killed preterminally, after 83 exposures, had extensive lobular pneumonia. Several dogs showed scattered hemorrhages in the mucosa of the gastrointestinal tract, and one surgically treated dog, killed after 24 exposures, showed several superficial mucosal ulcers in the pylorus.

The bone marrow showed increased cellularity, particularly evident in the epiphyses of the femur and tibia, in which the marrow is normally fatty. The arteries of the marrow often showed marked fibrous thickening of the adventitia. In two surgically untreated dogs exposed 96 times, some marrow spaces showed a network of cellular fibrous trabeculae, a few supporting large blood vessels (Fig. 11).

Scattered small hemorrhages were found in the brain and in the meninges. The usual absence of hemosiderin deposits and other changes in the parenchyma suggests that some of these hemorrhages were terminal or agonal. However, others probably oc-

curred before death. This is suggested by the presence of foci of demyelination in the corpus callosum of a dog with aortic insufficiency killed after 83 exposures. A control dog, killed after 96 exposures, showed a large area of softening of the cortex of a parietal lobe, with vacuolation of the parenchyma and dense infiltration by large mononuclear cells (Fig. 12). The superficial molecular layer was largely spared. Another control dog, killed after 44 exposures, showed a superficial area of softening along the ventral surface of the rostral part of the pons and severe encephalitis, with numerous widely scattered areas of neuronal degeneration and gliosis and perivascular collections of lymphocytes. Most of the other exposed dogs showed less marked changes in the brain, such as occasional small glial nodules and a few small perivascular collections of lymphocytes; similar lesions have been observed, but only occasionally, in unexposed dogs. Vacuolation of the nerve cells in the supraoptic nucleus and vacuolation and swelling of the ependymal cells forming the subcommissural organ were pronounced in several dogs exposed to hypoxia.

Comment

The presence of cardiac lesions, such as aortic and mitral insufficiency, does not reduce the acute tolerance of dogs to high altitude until levels of 34,000 to 36,000 ft. are reached. At these altitudes, 6 of 19 dogs with valvular lesions died, while only 1 of 15 controls failed to survive. Death was probably due to respiratory failure. One dog, which had stopped breathing at 36,000 ft., was revived by artificial respiration. This is in agreement with the findings in a similar altitude study at this laboratory,[4] in which electrocardiograms revealed that the hearts of dogs continued to beat (about 15 minutes) after respiration ceased; only occasionally was ventricular fibrillation observed.

The presence of scattered hemorrhages in various organs such as the brain have previously been reported in other animals [10] and in man [11] exposed to high altitudes. Recently the presence of fat emboli were reported [11] in the lungs of six of nine cases of decompression deaths in man. In our study the lungs of two of five acute altitude dogs studied for fat showed fat emboli in a few scattered alveolar vessels.

The altitude tolerance of dogs, even with cardiac lesions, is greater than that of other laboratory animals, such as the rat [12] and the cat.[13] It is remarkable that all of the dogs with valvular lesions and elevated left ventricular end diastolic pressures (Table 1) survived four hours at 30,000 and 32,000 ft. This is in agreement with the marked hypoxic resistance noted in dogs with extensive pulmonary resection [14] and with large myocardial infarcts.[4] However, it is important to note that one AI dog with endocarditis and myocarditis survived for only 15 minutes after reaching 30,000 ft. This suggests that resistance to altitude may be greatly reduced when valvular defects are associated with myocarditis or other severe pathologic lesions. Perhaps, similarly, the poor tolerance to hypoxia observed in patients with angina pectoris [3] may be due to an associated impairment of the myocardium, resulting from a relatively deficient coronary circulation.

When dogs with aortic insufficiency and controls not operated on were exposed repeatedly to 30,000 ft. simulated altitude over a prolonged period, there was no significant difference in their survival, in the degree of polycythemia produced, or in the severity of the pathologic changes found. The lesions tended to be severer and more varied than those reported in dogs exposed for a comparable period to 25,000 ft.[15] For example, renal and splenic infarcts, cardiac vegetations, and ulceration of the pylorus were found in dogs exposed to 30,000 ft. but were absent in dogs at 25,000 ft. Such lesions are attributable to high altitude, since similar lesions, even severer, were found in rats exposed to 25,000 ft. for longer periods.[16] In the rats the infarcts were considered to have resulted from

emboli derived from cardiac vegetations. However, among dogs exposed to 30,000 ft., only two of the seven with infarcts had cardiac vegetations. Perhaps such infarcts were due to fat embolism or to cardiac vegetations that had subsequently become completely organized or had undergone resolution. Another possibility is that thrombi, similar to those found in the lungs of two dogs, were formed in vessels of the kidney or spleen. Foci of softening and demyelination of the brain similar to those found in our dogs have been reported after hypoxia in other animals.[10] The formation of fibrous trabeculae in bone marrow, however, has not been reported previously.

Our findings indicate that high-altitude exposures are an important factor in the genesis of the arteriosclerotic lesions in the aorta of dogs, since lesions were found in young dogs, estimated to be under 2 years of age, and tended to vary in character and severity with the period of exposures to high altitude. In dogs given less than 30 exposures, the intimal plaques were thin, loosely cellular, and contained abundant material staining like acid mucopolysaccharide. In dogs exposed for longer periods, the plaques were usually much thicker (Fig. 8), were densely collagenized, and at times surmounted by a loosely cellular layer. The lesions in the aorta and in the myocardial arteries resembled in many respects those often found by Lindsay, Chaikoff, and Gilmore [17] in old dogs (8 to 14 years of age). While none of our dogs appeared old, the lesions, particularly in the myocardial arteries, tended to be less severe in young dogs than in more mature animals (estimated to be 4 to 7 years old) exposed for comparable periods. These findings suggest that age may have been a contributing factor in some dogs and, as reported previously in rats,[16] that prolonged exposures of dogs to high altitude hastens the development of degenerative lesions commonly associated with old age. One must consider the possibility that the lesions in young dogs elicited by the altitude exposures are due to hypoxia, or to the increased load on the vessels resulting from the polycythemia, or to a combination of these factors. The fact that lesions developed in one young dog after only 19 exposures before polycythemia became marked suggests that hypoxia is the most important factor. The fact that no such arteriosclerotic lesions were noted in dogs and rats with polycythemia exposed to 25,000 ft. for comparable periods [15,16] indicates that polycythemia is a less important factor, that there is a critical level of hypoxia below which lesions are not usually engendered, and that there may be a species difference in the character of the reactions to hypoxia. It is of interest that similar vascular lesions have been reported in a human case of pulmonary emphysema associated with severe hypoxia.[18] The findings in this clinical study and in the dogs of the present study are compatible with the theory that anoxemia is a causal agent in eliciting arteriosclerosis.[19]

Summary

Normal dogs and dogs with surgically induced aortic (AI) or mitral insufficiency (MI), or the two combined (AI-MI), received successive four-hour exposures to 30, 32, 34, and 36,000 ft. simulated altitude at 10-day intervals. The presence of these cardiac lesions does not reduce the tolerance to an acute exposure until levels of 34,000 to 36,000 ft. are reached. At those altitudes, 1 of 10 AI dogs died at 34,000 and 2 at 36,000 ft.; 1 of 5 MI dogs and 1 of 4 dogs with combined aortic and mitral insufficiency (AI-MI) died at 36,000 ft. Only 1 of 15 controls died at 36,000 ft. One AI dog, recovering from experimental endocarditis and myocarditis, died within 15 minutes at 30,000 ft.; this suggests a marked reduction in altitude tolerance when other severe pathologic lesions are present. Generally, death was due to respiratory failure. Pathologic studies revealed scattered hemorrhages particularly in the lung, brain, and endocardium. These studies show that dogs with cardiac valvular

lesions have a slightly decreased altitude tolerance, particularly above 32,000 ft.

Seventeen controls and five AI dogs were exposed six hours to 30,000 ft., four to five days weekly from 11 to 96 times. There was no significant difference in the survival of dogs operated on and dogs not operated on to the prolonged exposure. Both controls and AI dogs showed visceral engorgement, increased cellularity of bone marrow, focal endocardial and valvular thickening, focal myocardial fibrosis, aortic and occasionally coronary, nonlipid arteriosclerotic lesions, organizing cardiac vegetations, visceral infarcts, and renal hemosiderosis. One exposed dog showed focal demyelination of the corpus callosum, and another, softening of the parietal cortex.

Arteriosclerotic plaques were seen in the aorta in 12 dogs, including some under 2 years of age, and they varied in character with the number of altitude exposures. The lesions are attributed largely to hypoxia.

Mr. Milton Parker provided technical assistance in preparing blood pressure recordings.

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Fluorescent Stains, with Special Reference to Amyloid and Connective Tissues

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With the Technical Assistance of Rosemary Willins, B.A., R.T.

The purpose of this paper is to present an analysis and evaluation of basic fluorescent staining techniques, using a small ultraviolet light source and a routine microscope. This project was attempted in order to clarify the present position and potentiality of simple fluorescence microscopy in a routine histopathology laboratory. After review of the literature on this subject, it was found that no comprehensive analysis of practical fluorescent staining techniques and their results was available in English, although reports using these methods have appeared.

Several reviews [1-3] have covered many of the extensive aspects of fluorescence microscopy. The use of fluorescent stains in bacteriology is already well established,[4] and many papers are being published utilizing the fluorochrome-conjugated-antibody techniques. However, the use of direct fluorescent staining in the study of histological and histopathological material is less common. The use of acridine orange to demonstrate nucleoproteins [5,6] is of considerable interest, particularly applications of the technique in the field of exfoliative cytology [7,8]; an earlier investigation [9] utilized a combination of fluorochromes (berberine sulfate, acid fuchsin, and acridine yellow) for identification of malignant cells. A recent publication [10] reported the study of a variety of tissues, blood, and pigments using, mostly, acridine orange. Mucin has been shown [11] to exhibit fluorescence when stained with acridine orange. Several workers have used fluorochrome-conjugated proteins in direct histopathological studies—for example, as a plasma protein-tagging technique in investigation of liver damage,[12] using fluorescein and rhodamine B. The histology of the eye,[13] nerve tissue,[14] and skin [15,16] has been investigated with various fluorochromes. Another group [17] has demonstrated specific loss of fluorescence of malignant cells when stained with globulin-conjugated fluorescein; these workers have extended their studies to leukemic cells,[18] hemolytic anemia,[19] and the specific changes in fluorescence in hyperplastic and neoplastic diseases of the breast.[20] The demonstration of lipids [21,22] has become an established technique.

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The section on amyloid staining was presented at the 56th Annual Meeting of the American Association of Pathologists and Bacteriologists in Boston, April 23-25, 1959.

Methods and Materials

Apparatus.—Although many types of apparatus are described, often with complicated filter systems, it was decided to use a simple ultraviolet light source and a routine microscope, since the object was to determine what practical use this technique would have in a routine laboratory.

The unit used for all the experiments described was the Carl Zeiss small fluorescence equipment, a heat-absorbing filter, and an eyepiece-barrier filter (OG-5), which fits in the eyepiece, or directly over the prism in a binocular microscope. The lighting unit consists of a lamp housing, transformer, and stand, with a high-pressure mercury burner, OSRAM HBO 74,

which, together with a Schott BG-12 filter, gives ultraviolet light in the 350 mμ-450 mμ range, which is well within permissible limits. The use of copper sulfate to absorb light from the red end of the spectrum made little difference with the results obtained and has been discarded.

It is possible to use the Zeiss large fluorescent equipment which, having an OSRAM HBO 200 lamp, is approximately four and one-half times as bright as the OSRAM HBO 74 on the small equipment. This new lamp, in conjunction with two Schott BG-12 filters in the light source and a Zeiss OG-4 and Zeiss OG-5 filter in the eyepiece, gives a completely dark background with a great increase in resolution. This is not essential for all work, but is desirable. A front-surface-aluminized mirror is a slight asset, although not absolutely necessary.

The condenser in use is a four-lens aplanatic type, but almost any type of condenser may be used, and the cheaper two-lens Abbe type has the merit of absorbing less ultraviolet light than some of the more expensive, corrected types. Ordinary thin microscope slides and cover slips were used throughout. Slides should not have any tinge of green at the edges, since these are likely to be autofluorescent.

Tissues.—All sections used for the primary experiments were taken from routine blocks of tissue which had been fixed in formol-saline, processed through alcohol and chloroform, and embedded in paraffin. For each of these experiments, one section each of skin, kidney, appendix, and prostate was taken, other tissues that were appropriate being stained subsequently, depending on the results of the primary experiments. All positive results obtained were rechecked at later dates on different tissues.

Fixation.—As described above, all tissues used in the primary experiments were formol-fixed. It was found that, contrary to the impression given in most papers on the subject, fixation can be very important in specific instances. This was most noticeable when using acridine orange, since with formol-fixed tissue an indifferent picture was obtained; after fixation in alcohol a crisp differential picture in distinct shades of green and red was obtained; 10 minutes' treatment in formalin was sufficient to destroy this property in alcohol-fixed smears or tissue. Even with those staining techniques in which formalin fixation is adequate, or to be preferred, the duration of fixation should be kept as short as possible to avoid excessive autofluorescence of tissue.

Staining.—As a preliminary routine, sections were brought to water, stained for three minutes, rinsed in water, and mounted in buffered glycerin U.S.P. (pH 7,0).

Mordanting.—To see what effects those salts commonly used as mordants would have on fluorochromes, sections were pretreated in iron alum, phosphomolybdic acid, and phosphotungstic acid, with the results detailed in Experiments 5, 6, 7, 10, and 11.

Mounting.—It was decided, after the first experiment, to modify the accepted mounting technique, using buffered glycerin, since there was a great degree of diffusion of dye into the glycerin mountant, which gave specimens a colored halo and reduced, or even completely destroyed, any contrast present. Simple pressure on the cover slip immediately before examining slides reduced this to a great degree, but the impermanence of the mount led us to try Apáthy's medium as a mountant. This was a distinct improvement, there being minimal diffusion of dye into the surrounding mountant, and better resolution because of the higher refractive index of the mountant; furthermore, since Apáthy's medium sets rather hard, the need for ringing each cover slip is obviated. Control slides were examined in buffered glycerin to ensure that the Apáthy's medium did not interfere with staining; in Coons' fluorescent-antibody technique, it was found that the Apáthy's medium destroyed the specific fluorescence.

Fluorochromes.—The following dyes were labeled, as shown in the tabulation at the bottom of this page.

Staining Techniques.—It was decided to use each of the fluorochromes first, in a dilute aqueous solution, then in a very dilute aqueous solution, buffered in an acid buffer and then in an alkaline buffer, followed by alcoholic solutions of the dyes, and, lastly, to try the use of various mordants. These are listed as experiments, given in the order in which they are carried out. The results of these experiments may be seen in the accompanying Table.

Experiments

EXPERIMENT 1.—A simple 1% aqueous solution of each dye was prepared; sections were brought down to water, stained for three minutes, washed briefly, mounted and examined immediately.

EXPERIMENT 2.—The solutions used in Experiment 1 were further diluted to give a final concentration of 0.1% aqueous solution, sections again being stained for three minutes, rinsed in water, mounted, and examined immediately.

EXPERIMENT 3.—A 0.5% solution of each dye in sodium acetate-hydrochloric acid buffer at pH 3.95 was prepared and used as described in Experiment 1.

EXPERIMENT 4.—A 0.5% solution of each dye in sodium acetate-hydrochloric acid buffer at pH 8.6 was prepared and used as in Experiment 1.

EXPERIMENT 5.—Sections were immersed in 5% iron alum for 30 minutes, washed in running water for 5 minutes, and then stained as described in Experiment 1. Slides were examined immediately.

EXPERIMENT 6.—Sections were immersed in 5% phosphotungstic acid for 30 minutes, washed in running water for 5 minutes, and then stained as in Experiment 1. They were mounted and examined immediately.

EXPERIMENT 7.—This experiment was basically the same as Experiment 6, except that sections, after staining, were dehydrated in alcohol, cleared in xylene, and mounted in Harleco synthetic resin (HSR).

EXPERIMENT 8.—Sections were dewaxed in xylene, treated with alcohol for one minute, stained for three minutes in a 1% alcoholic solution of each dye, washed in alcohol, washed in water, mounted in Apáthy's medium, and examined immediately.

EXPERIMENT 9.—Similar to Experiment 8, but sections, after staining, were cleared and mounted in Harleco synthetic resin.

EXPERIMENT 10.—Sections were immersed in 5% aqueous phosphomolybdic acid for 30 minutes, washed in water for 5 minutes, then stained as in Experiment 1, dehydrated, cleared, and mounted in Harleco synthetic resin.

EXPERIMENT 11.—Similar to Experiment 10, except that, after staining, slides were mounted in Apáthy's medium direct from water.

EXPERIMENT 12.—Effects of oxidation: Sections were oxidized in 1% periodic acid for 10 minutes, then stained as in Experiment 1. After staining, sections were dehydrated, cleared, and mounted in Harleco synthetic resin.

In each case, sections were examined first in water, before being dehydrated, cleared, and mounted, to ensure that there

A. Rhodamine B
B. Phosphine 3-R
C. Thioflavin T
D. Rhodamine 3-GO
E. Uranin (fluorescein sodium)
F. Fluorescein
G. Atabrine (quinacrine)
H. Acridine orange
I. Titan yellow
J. Chlorophosphine
K. Acridine red
L. Magdala red
M. Acid alizarin blue
N. Acridine yellow
O. Acid fuchsin
P. Rhubarb extract
Q. Pyronin Y
R. Thionine
S. Acriflavine
T. Thioflavin S
U. Brilliant dianil green G
V. Auramin
W. Chlorophyll
X. Thiazine red
Y. Coriphosphine
Z. Berberine sulfate

	Exper. 1							Exper. 2							Exper. 3							Exper. 4						
	Aqueous 1%							0.1% Aqueous							0.5% in pH 3.95 Buffer							0.5% in pH 8.6 Buffer						
Tissue *	N	Cy	Co	Mu	E	R	M	N	Cy	Co	Mu	E	R	M	N	Cy	Co	Mu	E	R	M	N	Cy	Co	Mu	E	R	M
A	y	y		y	w				y		y	w			b							b						
B									p	y						b		w	w				b		w	w		
C	Y	y						Y	y	y					Y	y			w			y	y					
D				w	w	w				y	w	y	w													g		
E			y		w					y		w																
F	y		y		w			y		y		w			w		y		w			w				w		
G	y	y			y	y		y	y				Y	Y	Y						Y			Y		Y	y	Y
H	G	r	g	g	y		g	G	r	g	g	y		r	G	r										Y		
I	w				W			w				W										w	w			W		
J					w							w																
K	O							O							O	w						P	w					
L	b	w				w		b	w				w			O		o	W	w	G					W		
M					w							w														w		
N	Y	y	Y	y				Y	w	Y	y				Y	y												
O																						w				w		
P					w							w							w							w		
Q		o	w						o	w					w				w									
R																												
S	y	y						y	y						y	g			g			o		g			g	
T																												
U	w							w																				
V	y	g	g	y	y			y	g	g	y	y			Y														
W					w							w							W			w				w		
X	w	w			O			w	w			O										W						
Y	O	g						O	g						o			g				r		g	o			
Z	y	y	Y		Y			y	y	Y		Y			Y	y	y		y			y	y	G		y		
Z (Cleared)	Y	y	Y		Y			Y	y	Y		Y			y	y	y		y			y	y	y	y	Y	y	

	Exper. 5							Exper. 6							Exper. 7							Exper. 8						
	After Treatment with 5% Iron Alum							After Treatment with 5% Phosphotungstic Acid							5% Phosphotungstic Acid Cleared							1% Alcoholic Stain Water Mount						
Tissue *	N	Cy	Co	Mu	E	R	M	N	Cy	Co	Mu	E	R	M	N	Cy	Co	Mu	E	R	M	N	Cy	Co	Mu	E	R	M
A	r									r												w				w		
B			g					y		g					y				g									
C					w			Y	g						Y	g			Y			W						
D					w			y		r		y			g				g									
E			y	g													g	w		w								
F								Y	b	y					w					w							y	
G					w		O	G	r			g			g							y						
H																						g	r			g		y
I	y	y	y	g		y						w					y	g		W			Y		Y		
J			y		y			r														w				W		
K																	r											
L		r	r		O																							
M																												
N	y				Y		y	Y	b						y							y						
O																												
P					W		w					w			g							w						
Q																												
R	y											g			Y				y			y						
S	y		y	g											g				w	w						Y		
T					W			Y	y						y							w						
U																						y						
V																												
W			r		w																							
X	g							r																				
Y	y	y	y	y	Y			Y				g			w							w						
Z	y	b			y																	Y	y	Y	w	Y		
Z (Cleared)																												

	Exper. 9							Exper. 10							Exper. 11							Exper. 12						
	1% Alcoholic Stain Cleared							5% Phosphomolybdic Acid Water Mount							5% Phosphomolybdic Acid Cleared							1% Periodic Acid and 1% Alcoholic Stain						
Tissue *	N	Cy	Co	Mu	E	R	M	N	Cy	Co	Mu	E	R	M	N	Cy	Co	Mu	E	R	M	N	Cy	Co	Mu	E	R	M
A			g		G	g				r									R			r	y	g	g		o	
B	w		y		y			y							y				Y			g	b	y	y		y	
C	g		y	y		Y		y							y							y	y					Y
D																			g			g						
E				g	g												Y			Y		y	g		g	y		
F	y					Y		y							w				W			y						
G			y			w		y																				
H	g					g		r											g							o		
I	y	y	y			Y		y		g			y			g	g	g		w						y		
J																												
K																							r					
L			r		O												r					w		r				
M																												
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Z	y	y	Y	y	Y			y														Y						

* Tissue code: N indicates nucleus; Cy, cytoplasm; Co, collagen; Mu, muscle; E, elastic; R, reticulin; M, mucin.
† Color code: B indicates brown; G, green; O, orange; P, pink; R, red; W, white; Y, yellow.

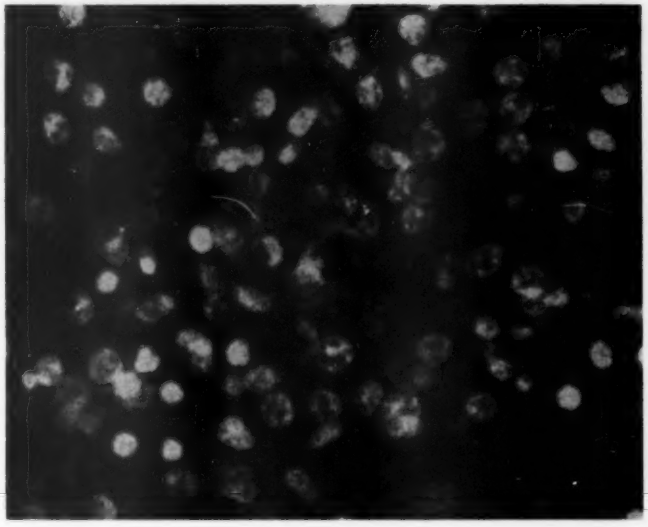

Fig. 1.—Nuclei stained with thioflavine S.

was no basic difference in the staining, except where the duplicate experiment was being carried out.

Results

Nuclei (Fig. 1).—Several of the dyes tested appear to be specific for deoxyribonucleic acid (Table), since the fluorescent staining pattern is almost identical with that seen with Feulgen's reaction. Acridine orange, as reported by Armstrong,[5] has already been shown to be differentially specific for both DNA (green) and ribonuclei acid (RNA) (red).

Cytoplasm.—Only stains H (acridine orange) and Y (coriphosphine) gave a color differentiation between nucleus and cytoplasm; and, since H gave such an excellent result, it is recommended as a general cytological fluorescent stain.

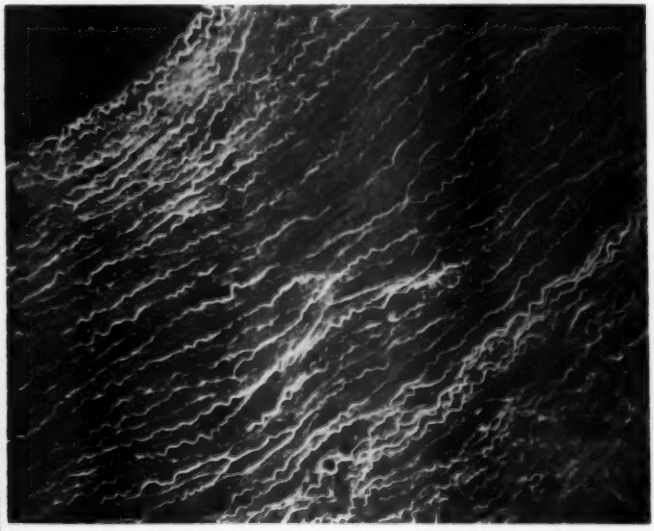

Fig. 2.—Elastic tissue in artery stained with acriflavine.

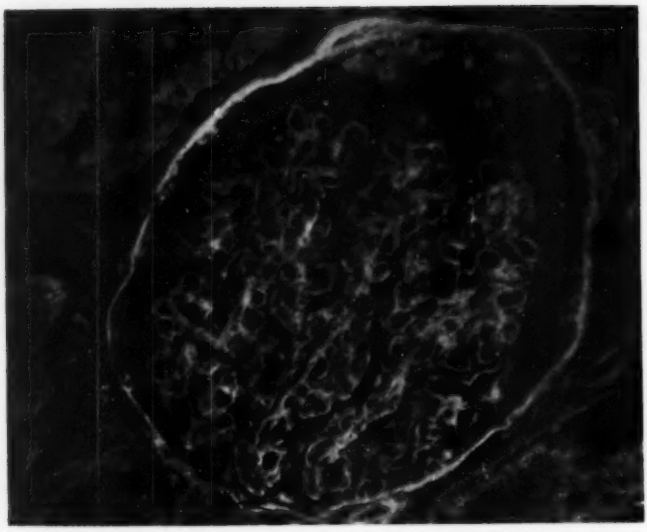

Fig. 3.—Basement membrane of glomerulus stained with rhodamine B.

Elastic Fibers (Fig. 2).—These fibers are autofluorescent to a great degree. Unstained or hematoxylin-and-eosin-stained sections show elastic fibers very well when viewed under the fluorescent microscope. They show exceptionally well in cleared sections after alcoholic acriflavine staining. The absolute specificity of this stain has yet to be tested.

Collagen and Muscle.—None of the dyes tested under the conditions described has given a good differentiation of connective tissue. However, a mixture of equal parts of 1% alcoholic solutions of rhodame B and fluorescein, differentiated with 1% acetic alcohol, dehydrated, cleared, and mounted in HSR, gave excellent, differential fluorescent staining of basement membrane, reticulin, collagen, and osteoid tissue.

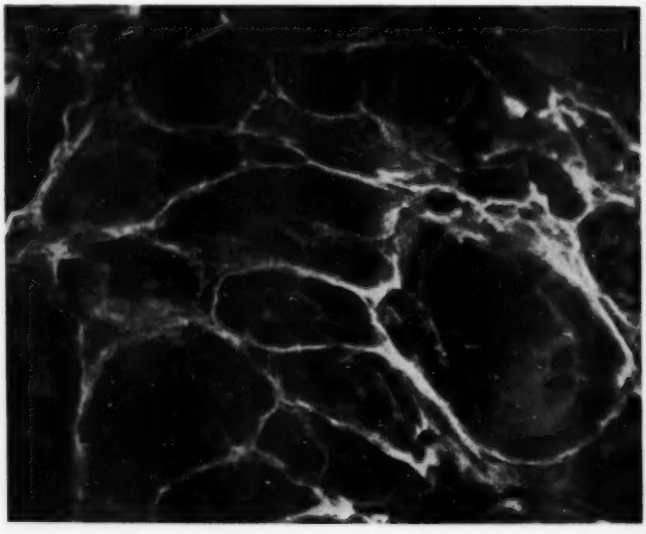

Fig. 4.—Basement membrane of thyroid acini stained with rhodamine B.

Mucin.—The staining of mucin, using acridine orange, was described by Hicks,[11] who, working simultaneously, discovered that pretreatment of tissue with iron alum yielded a specific stain. We became aware of this fact after Experiment 5, when sections stained with acridine orange showed mucin as bright orange; unfortunately, the color disappears on prolonged examination with ultraviolet light. Mucin in sections stained with Atabrine in simple aqueous solution at pH 3.95 and pH 8.6 buffer gave a bright yellow fluorescence, which did not fade. Specific fluorescence is inhibited by Apáthy's medium, and sections must be mounted in glycerin. The best result was given by the dye in pH 8.6 buffer. Magdala red in pH 3.95 buffer showed mucin as light green.

Reticulin (Figs. 3 and 4).—Several stains demonstrated basement membrane and reticulin (Table), but the best demonstration was given in cleared sections after alcoholic staining, noticeably with fluorescein and rhodamine B. This staining is seen to advantage in sections of thyroid gland, where dissolution of basement membrane is easily seen in Hashimoto's thyroiditis; kidney sections exhibit typical basement membrane thickening in membranous glomerulonephritis. This technique is simple to apply, and certainly has a less vigorous effect on sections than the sulfation technique.

Amyloid (Figs. 5, 6).—Since there is no satisfactory method of demonstrating amyloid in paraffin-embedded tissues, it was decided to utilize fluorochromy for this purpose. Sections from an amyloid kidney were stained with the 1% aqueous solution of all the dyes available. It was found that in sections treated with Stains C, H, N, S, and Y areas showing amyloid could be differentiated from surrounding areas by their marked fluorescence, stain C (thioflavin T) being by far the best. This effect was enhanced by differentiation in 1% acetic acid for 10 minutes, which reduced the fluorescence of normal tissue without affecting that of the amyloid. In some tissues it may be necessary to quench nuclear fluorescence by prestaining sections in alum hematoxylin for two minutes; no differentiation of hematoxylin is necessary.

Tissues from several different cases of amyloidosis were stained (1% aqueous thioflavine T, differentiated in 1% acetic acid for 10 minutes, washed, and mounted in Apáthy's medium) by this method with success. Attempts to differentiate between primary and secondary amyloidosis by

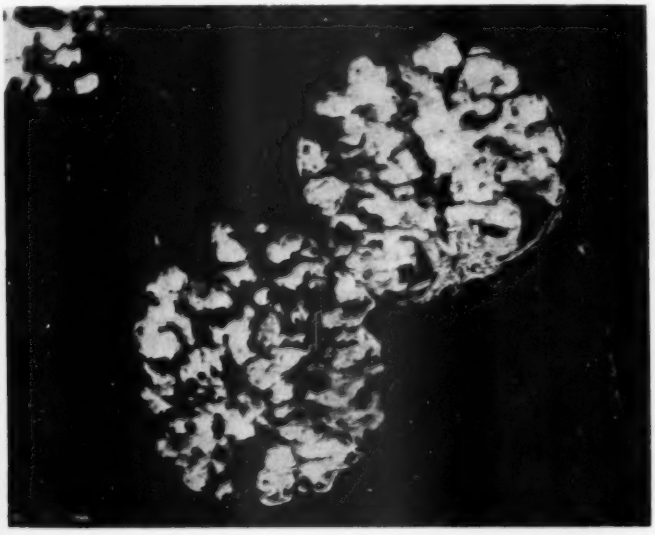

Fig. 5.—Secondary amyloid in kidney, stained with thioflavine T.

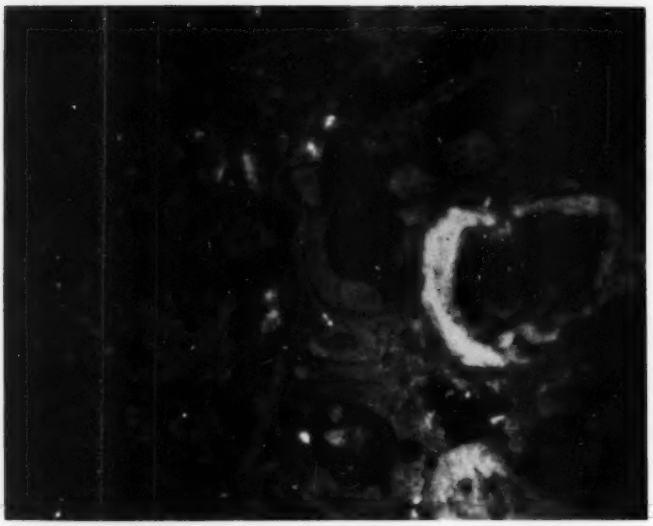

Fig. 6.—Primary amyloid in kidney, stained with thioflavine T.

staining methods were made, and it appeared that primary amyloidosis gave a slightly brighter fluorescence. Some slight difference was observed, however, in unstained sections, where areas of primary amyloidosis had a very slight yellow autofluorescence, compared with little or none in cases of secondary type. Blocks from five cases of primary amyloidosis, many cases of secondary amyloidosis, and sections of kidney from cases of diabetic glomerular sclerosis, arteriolar nephrosclerosis, membranous glomerular nephritis, and chronic glomerular nephritis were then examined to determine whether or not this method would differentiate between them and amyloidosis. In every section, except for amyloidosis, glomeruli were negative. Sections from cases of chronic glomerular nephritis and arteriolar nephrosclerosis showed some fluorescence in the tubules; but in each case this was of a granular nature and not to be confused with the amorphous nature of amyloidosis. Differentiation between amyloidosis and the above conditions presented no difficulty. Recently a biopsy specimen of skin was received from a case which gave very poor staining reactions with methyl violet and Congo red, yet gave the slight suggestion of amyloidosis in hematoxylin-and-eosin-stained sections. Sections stained with thioflavine T gave a very strong positive result, and sections from previous biopsy tissue from the same patient were then recut and stained; these were strongly positive. A diagnosis of primary amyloidosis was made on these sections, stained and unstained. Two months later the patient died and showed generalized primary amyloidosis. Fluorochrome staining has now been adopted as a routine for the diagnosis and demonstration of amyloidosis in this laboratory.

Comment

The results we have so far obtained with these staining techniques have been encouraging. There appears to be as high a degree of specificity of staining with fluorochromes as is obtainable by conventional staining methods; moreover, they appear to be simpler in operation. Variations in fixation, mordanting, buffering, and mounting play as important a role as in standard staining techniques; this is well illustrated by the variations in staining reactions under the different conditions seen in the Table. Combinations of stains, while not

completely successful so far, have given some promising results. For example, a combination of acridine orange and berberine sulfate gave a brighter nuclear picture, with improved definition, than either stain alone.

Of the stains described, we feel the following may justify adoption as alternatives to routine methods: Thioflavine T for demonstration of amyloid, it being far superior to either Congo red or methyl violet; fluorescein and rhodamine B for reticular and osteoid tissues; acridine orange for nucleic acids instead of the Unna-Pappenheim method; acriflavine for enhancement of autofluorescence of elastic fibers of ultraviolet microscopy of a routine hematoxylin-eosin section; mucin stained by acridine orange or Atabrine, their merit being simplicity of preparation and use of the stain.

Summary

This paper presents an account of various experiments designed to assess the value of simple fluorescent staining techniques, using a small ultraviolet light source.

There appear to be several simple fluorescent staining techniques available for selectively demonstrating the presence of mucin, elastic tissue, basement membrane, reticulin, nucleoproteins, and amyloid. Doubtless, many other specific staining characteristics of numerous tissues remain to be identified. Several of these simple histological techniques have some distinct value as routine or research methods.

We wish to record our gratitude for the encouragement and advice given us by Prof. H. E. Taylor and Dr. H. K. Fidler, Vancouver, B.C., Canada.

Vancouver General Hospital (9).

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A New Silver Method for the Golgi Apparatus

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As a result of previous studies regarding the influence of the pH of fixatives, as well as components of the fixative mixtures, on the staining of tissues, a fixative composed of glycine (aminoacetic acid), HCl, and formalin was recently proposed for improved staining of neuroglia with del Rio Hortega's silver carbonate and Ramon y Cajal's gold-sublimate methods.[1] In the course of the experiments it was observed that the same fixative could be used for the silver staining of the Golgi apparatus, though modified as follows: 1. The HCl was substituted by acids which do not precipitate silver salts, such as HNO_3, HF, and H_2SO_4. 2. It was found that the best pH of this fixative for the silver staining of the Golgi apparatus was approximately 2.9. In general, if the pH is raised above 2.9, a more intense staining of the Golgi apparatus is obtained; but at the same time other structures are stained which tend to mask it. On the other hand, if the pH is below 2.9, the background is more transparent, but the Golgi apparatus is stained more weakly. 3. The proportion of glycine in the mixture is of importance: It should contain a suitable quantity, and this has been found through trial and error.

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Taking these considerations into account, the following formula was finally obtained:

Glycine	1.7 gm.
1 N nitric acid	4.6 ml.
Formalin	15 ml.
Distilled water to make	100 ml.

The variability in the fixation time of different tissues in order to obtain a good staining of the Golgi apparatus is well known. This inconvenience is not avoided with the fixative proposed herein, and certain experimentation may be necessary to attain the exact time with any given type of tissue. In general, this varies from 3 to 24 hours. In some tissues the Golgi apparatus is very difficult to stain, perhaps because it requires a very exact fixation time or because of other, unknown factors. As with other fixatives, it is essential to fix extremely fresh tissues.

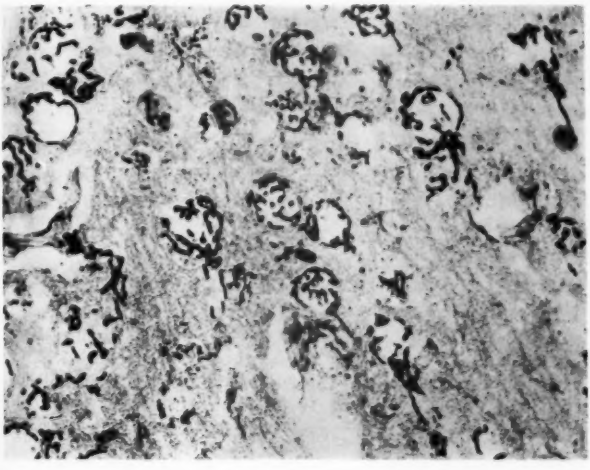

Fig. 1. — Golgi apparatus stained with the author's silver method without gold toning in the cortical cells of the cerebrum of a guinea pig. Fixation time five hours; × 600.

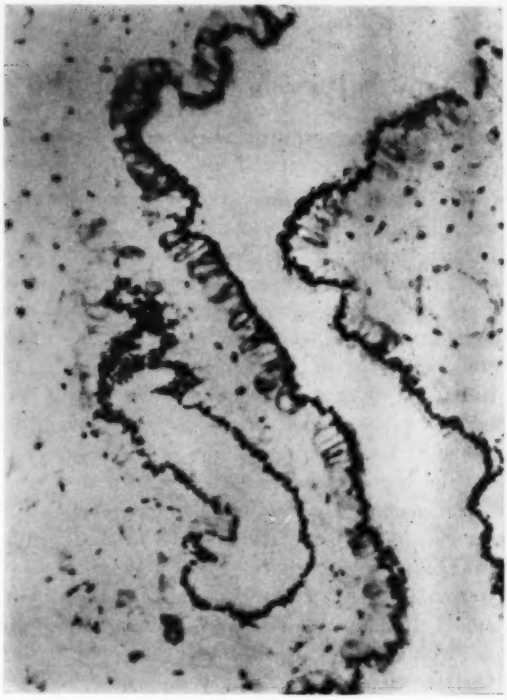

Fig. 2.—Golgi apparatus stained with the author's silver method with gold toning in the glandular cells of a human uterine mucosa. Fixation time four hours; × 300.

After the fixation, the small blocks of tissue should be briefly washed in distilled water and placed in 1.5% silver nitrate for 24 hours. Then, they are rinsed in distilled water and immersed in Cajal's developer (hydroquinone-formalin) for 12 to 24 hours. The tissues are washed in distilled water and frozen sections cut; or they are dehydrated with alcohols, cleared, and embedded in paraffin. Gold toning of the sections is optional.

Comparative studies (man, guinea pig, rabbit, rat) showed that with the fixative proposed herein, Golgi apparatus can be stained with more intensity than by using Cajal's fixative, and that the fixative can be applied to a greater variety of tissues. It is superior to da Fano's fixative with regard to the nervous tissue, and in other tissues the background is more transparent and Golgi apparatus brighter. With Elftman's method,[2] which is theoretically interesting, we have obtained unsatisfactory results: Golgi apparatus was stained with poor precision or was not stained at all, and the background presented a coarse precipitate.

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Influence of Anoxia and Muscular Contraction upon Myocardial Glycogen in the Rat

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It is generally agreed that the myocardium of experimental animals becomes depleted of glycogen in the course of coronary artery ligation, systemic anoxia, and postmortem autolysis.[1-10] Application of the periodic acid-Schiff (PAS) technique to tissue sections [11-13] has added to these quantitative studies such qualitative information as is afforded by histotopographical analysis. Nonetheless, knowledge is still incomplete with respect to comparative rates and anatomic patterns of glycogen depletion under the above experimental conditions.

In the current study, to prevent glycogenolysis during the process of obtaining the myocardial specimens,[6,14] artificial respiration was maintained until removal of the hearts was completed; myocardial standstill was produced by transventricular ablation (below rather than through the atrioventricular groove), which caused abrupt cessation of contraction. This combination of techniques yielded specimens which were considered equivalent to biopsy tissue of the living, fully oxygenated heart.

The methods of coronary artery ligation in the dog commonly employed for the metabolic investigation of ischemic myocardium have been criticized on the grounds that an infarct so produced is a heterogeneous mixture of unaltered, injured, and necrotic fibers.[15] The technique of coronary artery ligation in the rat by Johns and Olson [16] has provided a tool for producing large infarcts which not only are homogeneous but can also be studied in their topographical relationships by single cross sections comprising the entire ventricular apparatus.

In the series of experiments here reported attention was focused on the following determinations: (1) glycogen content of normal myocardium; (2) comparative rates of glycogen depletion incident to postmortem autolysis, systemic anoxia, and coronary artery ligation; (3) changes in topographical distribution of glycogen during postmortem autolysis, systemic anoxia, and coronary artery ligation, and (4) identification of other factors influencing glycogen depletion of the myocardium.

Materials and Methods

Adult white rats of the Charles River and Sprague-Dawley strains, weighing 150-250 gm. and divided equally between males and females, were used. The animals were kept at room temperature and fed Purina Laboratory Chow. Water was available ad libitum. Ether anesthesia was employed for all procedures. For artificial respiration oxygen was administered by face mask under intermittent positive pressure. Animals allowed to survive for more than six hours were returned to their preoperative conditions.

Group I (21 animals).—This experiment was designed to study the effects of postmortem autolysis upon glycogen content of the myocardium. The heart was exposed through a left anterolateral thoracotomy at the level of the fourth or

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A preliminary report of this study was presented at the 54th Annual Meeting of the American Association of Pathologists and Bacteriologists, Washington, D. C., April 11-13, 1957.

From the Departments of Pathology, the Bronx Hospital, and Albert Einstein College of Medicine of Yeshiva University, New York, and the Departments of Surgery and Pathology, Beth Israel Hospital and Harvard Medical School, Boston.

fifth intercostal space. Throughout the procedure anoxia was prevented by artificial respiration. After pericardiotomy, the ventricles were exteriorized with the aid of abdominal pressure. Specimens were obtained by transverse ablation of all but the basal one-fourth to one-third of both ventricles. The ablated specimen was sliced transversely into three equal portions. The middle slice was placed immediately into cold fixative and served as control. The other two slices were returned to the chest cavity, where they remained immersed in accumulated blood, at room temperature, for varying intervals before being transferred to the cold fixative.

The choice of transventricular ablation as against ablation through the atrioventricular sulcus was based on the observation that with the latter technique the myocardium continued beating for several minutes, whereas with the former technique cardiac action ceased almost instantaneously.

Group II (23 animals).—This experiment was designed to study the effects of systemic anoxia (due to apnea) upon glycogen content of the myocardium. Bilateral pneumothorax was induced by removal of the anterior chest wall, no artificial respiration being applied. After a few seconds of intense cyanosis, the myocardium turned ashen-gray, beat with decreased vigor, and displayed progressive bradycardia, the normal rate, of about 350 a minute, dropping to 180 after 1 minute, 50-60 after 5 minutes, and 1 after 10 minutes. Specimens were obtained by transventricular ablation exactly as in Group I, though without prior pericardiotomy. The ablations were done immediately after thoracotomy and at varying times thereafter for periods up to 22 minutes, but always prior to cardiac standstill. The specimens were triple-sliced and treated like those of Group I.

Group III (36 animals).—This group served to study the effects of coronary artery occlusion upon glycogen content of the myocardium. The left coronary artery was ligated according to the procedure of Johns and Olson,[16] which, in brief, included artificial respiration, left anterolateral thoracotomy, pericardiotomy, exteriorization of the heart, and ligation of the left coronary artery (usually including the vein) with 000000 silk on an Atraumatic needle. Within a very few contractions, a territory of varying size of the left ventricle became intensely cyanotic. The degree of alteration of contraction within the cyanotic territory could not be determined visually. Unless the animal was to be killed in less than five minutes, the chest was closed in two layers. There was an immediate mortality rate of approximately 30%. The 36 surviving animals were killed in from 2 minutes to 10 days after coronary artery ligation and utilized for histochemical investigation. As controls for this group, two additional animals were subjected to a sham operation, during which the ligature was placed, but not tied, around the coronary artery. Specimens were obtained by transventricular ablation with the animals receiving artificial respiration, and processed as in the previous groups. Only the middle slice was studied.

All tissues were fixed in refrigerated Rossman's solution[17] and kept at 4-8 C for 24 to 48 hours. The blocks were washed in several changes of absolute alcohol, cleared in cedarwood oil and xylene, and embedded in paraffin. A minimum of five consecutive sections were stained as follows: (1) hematoxylin and eosin; (2) periodic acid-Schiff method of McManus, (*a*) counterstained with hematoxylin, (*b*) not counterstained, and (*c*) preceded by digestion with saliva or diastase for one hour at room temperature, and (3) McManus routine, as in (2), but omitting oxidation with periodic acid. Sections stained by methods *2a*, *2b*, and 3 were coated with celloidin after deparaffinization.

Glycogen was defined as that granular substance which stained purple with the periodic acid-Schiff method of McManus, which was digestible with human saliva or malt diastase (pH 7.2) in one hour at room temperature, and which did not give the Schiff reaction without prior oxidation with periodic acid.

Best's carmine stain, where used parallel with the periodic acid-Schiff routine, gave identical results.

Glycogen was recorded in terms of quantity (0 to 4+) and topographical distribution.

Results

The myocardial slices of the Group I animals placed in fixative immediately after transventricular ablation served as controls for all groups. These slices contained maximal quantities (4+) of stainable glycogen throughout both ventricles, including the septum. The intracellular distribution varied, depending on the distance from the natural surfaces: In the middle layers

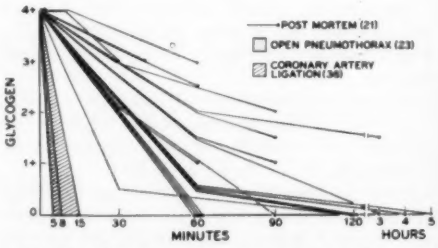

Fig. 1.—Diagrammatic representation of rate of glycogen depletion in the myocardium of rats under various conditions. For details see text.

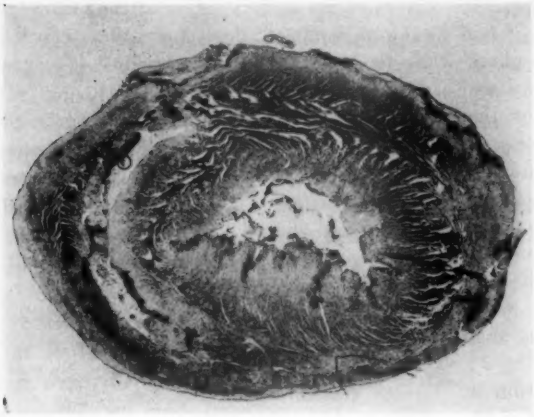

Fig. 2.—Total ventricular cross section of the heart, 40 minutes post mortem (Group I animal). There is equal depletion of glycogen in the two ventricles, the loss being more advanced beneath the natural surfaces than in the middle mural layers. In this, and in other photomicrographs, the left ventricle is on the right-hand side of the pictures. PAS stain without diastase digestion; no counterstain; × 7.5.

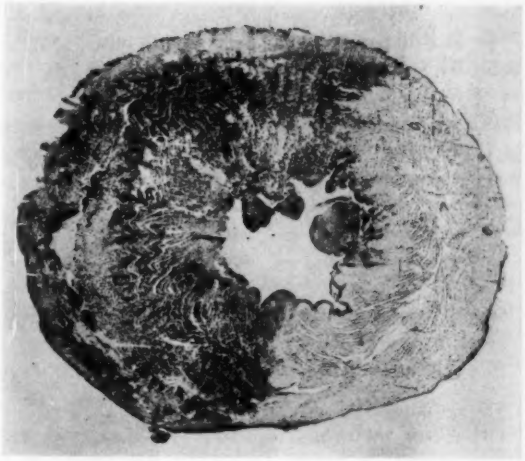

Fig. 3.—Myocardial infarct of left ventricle six hours after coronary artery ligation (Group III animal). A sharply defined segment of the free left ventricle is completely deglycogenated with the exception of a narrow subendocardial layer and of the papillary muscle. The infarct displays nondigestible PAS positivity. A pale zone, totally PAS-negative, can be recognized close to the endocardium and along the lateral borders of the infarct. PAS stain without digestion; no counterstain; × 7.5.

Fig. 4.—Myocardial infarct of left ventricle five minutes after coronary artery ligation (Group III animal). Glycogen in the ischemic territory is markedly reduced, but least so in the central portion. PAS stain without digestion; no counterstain; × 7.5.

of the wall glycogen was distributed evenly within the myofibers, but elsewhere it had drifted toward the sarcolemma in a direction antipodal to the endocardium and epicardium, respectively. Delayed fixation of the slices was associated with progressive diminution of glycogen, as shown in Figure 1. In the majority of specimens, deglycogenation required one to two hours, although occasionally glycogen remained demonstrable for longer periods. The rate of depletion was slower in the middle mural layers of both chambers and septum than in either their inner or outer third (Fig. 2).

The hearts of the Group II animals, beating under anoxic conditions (apnea), lost their glycogen rapidly; from 50% to 75% had disappeared by one to three minutes. By five minutes no more than a trace of glycogen was demonstrable in an occasional specimen, and no heart showed any glycogen after eight minutes (Fig. 1). The loss tended to be speediest in the middle layers of the wall. Ablation of the ventricles prior to complete deglycogenation retarded the depletion rate of the residual glycogen at least tenfold.

Following ligation of the left coronary artery in the Group III animals, depletion of glycogen was evident by two minutes after occlusion, i. e., in the earliest of preparations which could be secured; depletion was complete in from 5 to 15 minutes. Deglycogenation was confined to a sharply delineated, transmural territory, which always included part or all of the free left ventricle (Fig. 3). Glycogen depletion was more rapid about the periphery of the ischemic zone and slower in its center (Fig. 4). The papillary muscles, interventricular septum, and right ventricle were involved, in decreasing order of frequency. The size of the depleted territory bore no relation to the time which elapsed between coronary artery ligation and the

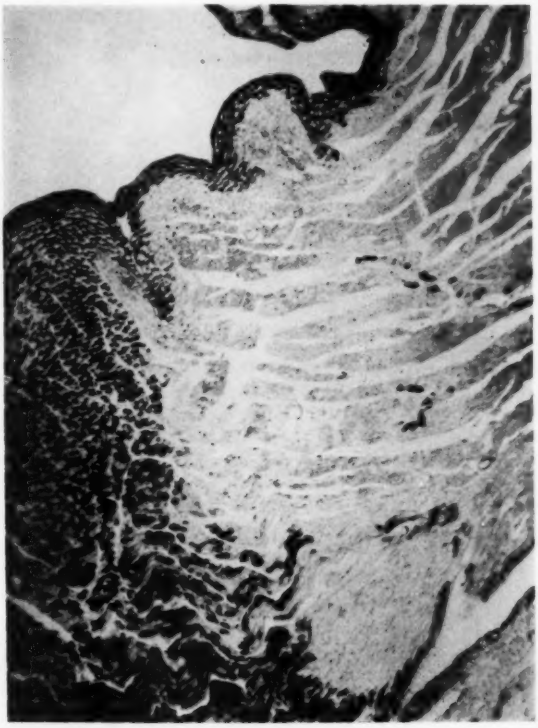

Fig. 5.—Myocardial infarct of left ventricle, six hours after coronary artery ligation from same specimen as that illustrated in Figure 3. The subendocardial myocardium is richly glycogenated. Note multiple examples of paravenous preservation of glycogen, best shown at the bottom of the photograph. A PAS-negative zone separates the glycogen-rich, viable myocardium on the left from the PAS-positive, but glycogen-free, infarcted myocardium on the right. PAS stain without digestion; no counterstain; × 50.

killing of the animal. Late preparations never showed evidence of reglycogenation.

An almost constant finding in the Group III animals was the persistence of glycogen in a narrow layer of myocardium situated just beneath the endocardium, the "subendocardial myocardium" (Fig. 5). Persistence of glycogen was also frequently observed (*a*) in small islands just beneath the epicardium and (*b*) in narrow perivenous collars of myocardium within the ischemic territory (Fig. 5). The myocardium outside the area supplied by the ligated left coronary artery did not differ in its glycogen contents from the control animals of either Group I or Group III. At no time was there an apparent increase of glycogen around the ischemic fields such as has been observed in human [18,19] and canine [11] hearts.

After the glycogen had disappeared from the ischemic myocardium, and beginning at about 30 minutes following coronary artery ligation, the cytoplasm of the myofibers developed a diffuse, nongranular, PAS reaction which—unlike glycogen—was not digestible with saliva or malt diastase, but which—like glycogen—failed to appear if oxidation with periodic acid was omitted from the McManus routine (Fig. 5). An intensified pink staining reaction equally resistant to diastase digestion was observed in sections stained with Best's carmine method. As the period of ischemia lengthened, both staining reactions became intensified. Once they had reached a maximum, they remained so until the necrotic muscle disappeared.

The glycogen-free, PAS-positive, ischemic territory was bordered at its sides and subjacent to the "subendocardial myocardium" by a narrow, pale zone unstained with the PAS technique (Fig. 5). Topographically, this pale border coincided with that peripheral zone of the ischemic territory which earlier had been observed to become depleted of glycogen most rapidly. About four hours after coronary artery ligation, i. e., at the time when infarction became recognizable in hematoxylin-eosin-stained sections, the pale zone subjacent to the "subendocardial myocardium" developed diastase-resistant PAS positivity, like that already present. The border zone along the lateral edges remained pale for another 24 to 48 hours, when—incident to the evolution of marginal granulation tissue and repair—it, too, became lost as a distinct layer.

Comment

Glycogen in the rat heart was studied histochemically in relation to postmortem autolysis, systemic anoxia, local ischemia, and in situ cardiac action. Postmortem autolysis (Group I animals) was considered the equivalent of glycogen depletion under conditions of complete anoxia and suspended muscular contraction. Following sudden cessation of heart beat induced by transventricular ablation, disappearance of glycogen by autolysis required from one to two hours or more. By contrast, sustained muscular contraction in the face of systemic anoxia (Group II animals) caused the glycogen to disappear many times faster, usually within a matter of five minutes. Evans [6] reported similar results in the intact rat after clamping of the trachea. Glycogen within the territory supplied by a ligated coronary artery disappeared at a rate comparable to that of the Group II animals. This suggested that the ischemic part of the ventricular muscle continued to contract for some period. Although visual inspection of the rat heart was not reliable on this point, observations in the dog indicate that muscular contraction in the ischemic territory does not stop instantaneously but continues for a minute or so.[20]

The decisive influence which muscular contraction has upon glycogenolysis was illustrated by the marked slowing in the rate of depletion whenever the beating heart of the apneic animal was brought to abrupt standstill prior to complete disappearance of glycogen. The chronological parallelism between rate of glycogen depletion during

anoxic contraction and progressive severity of bradycardia agrees with physiological concepts that glycogen is the foodstuff par excellence for the production of rapid energy. According to Evans,[6] cardiac contraction ceases when glycogen becomes reduced by more than 80% of normal in rats kept in a hypoxic atmosphere.

Chemical data on glycogen depletion of the rat's myocardium beating in situ under anoxic conditions [6,7,10] are in agreement with the histochemical observations here recorded. By comparing hearts beating in situ with those after extirpation, Bloom [10] concluded that it is not cardiac contraction which is the essential mechanism of glycogen depletion but cardiac work against peripheral vascular resistance. If so, the terms "muscular contraction" and "cardiac contraction" as here used should be read so as to include cardiac work.

The left ventricle of the rat heart is supplied by a single (left) coronary artery, which appears to be an end-artery.[10] Within minutes, its ligation is followed by deglycogenation in a territory conforming topographically with the infarct which by ordinary histopathologic criteria (smudginess of fibers, inflammatory reaction, etc.) will not become demonstrable until four hours later. Hence, it is inferred that loss of glycogen after coronary ligation is a very early indicator of infarction. Histochemical observations on the activities of DPNH-diaphorase (reduced diphosphopyridine nucleotide-diaphorase), lactic dehydrogenase, and ATP-ase (adenosine triphosphatase) in the ischemic myocardium of the rat disclose that glycogen depletion long precedes any demonstrable changes in the activities of these enzymes.*

In the distribution area of the ligated coronary artery, transendocardial alimentation was thought to be responsible not only for the continued structural survival of a narrow subcavitary layer of myocardium but also for the persistence of its glycogen. Thus, the disappearance of glycogen from this zone which followed oxygen reduction of the ventricular blood during apneic anoxia was as anticipated. By inference, the preservation of glycogen around coronary veins within the territory of a ligated coronary artery suggested retrograde blood flow from neighboring, normally oxygenated myocardium. Hence, in preventing deglycogenation, the availability of oxygen rather than any intrinsic properties of certain fibers (Purkinje fibers) appeared to be the determinant factor.

Summary

As an experimental model for histochemical investigation of the myocardium a technique was desired which would preserve the metabolic status of the living tissues. This was accomplished by ablation of the ventricles below rather than through the atrioventricular groove, a technique which causes instantaneous arrest of cardiac contraction and which, in combination with artificial respiration during thoracotomy, yielded specimens considered equivalent to biopsy tissues of the living, fully oxygenated heart.

Application of this technique in the rat has yielded the following findings:

(*a*) Normal (ventricular) myocardium in vivo is rich in glycogen.

(*b*) Depletion of myocardial glycogen by postmortem autolysis requires one to two hours or more. By contrast, deglycogenation is complete within a matter of minutes when the heart is beating in situ under conditions of systemic (apneic) anoxia. This difference suggests that muscular contraction (in the intact animal) critically influences the rate of glycogen depletion of the myocardium during anoxia.

(*c*) The myocardium deprived of its blood supply by coronary artery ligation becomes deglycogenated within 5 to 15 minutes, i. e., within a time interval comparable with that of anoxically contracting myocardium. This rapid depletion appears

* These examinations were done by Dr. Alex B. Novikoff, whose cooperation is gratefully acknowledged.

to be a more sensitive indicator of incipient infarction than the histochemically demonstrable changes in the activities of several enzymes (DPNH-diaphorase, lactic dehydrogenase, and ATP-ase).

(*d*) In preventing deglycogenation, the availability of oxygen rather than any intrinsic properties of certain fibers (Purkinje fibers) appears to be the determinant factor.

The Bronx Hospital, Fulton Ave. at 169th St. (56).

Dr. George W. Curtis gave valuable advice. Mr. Stanley R. Waine, medical artist, executed Figure 1.

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Differential Staining of Normal and Neoplastic Tissue with Fluorescein-Egg Albumen

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Introduction

Since the demonstration of differential staining of normal rat liver and rat hepatomatous tissue,[1,2] using fluorescein-conjugated organ-specific antisera, it has been shown that fluorescein conjugates of normal (nonimmune) rabbit serum [4] and of various serum proteins of a diverse series of animals [5,6] will produce identical differential staining of a wide variety of tumors. This clearly indicates that the differential staining is an immunologically nonspecific phenomenon; indeed, it has been shown to be a physiochemical protein-protein interaction.[3] A natural extension of this study was the investigation of proteins other than those stained from serum. In this communication the preparation and staining affinities of fluorescein isocyanate-egg albumen conjugates are described.

Methods

Fluorescein isocyanate was conjugated with egg albumen by the methods of Coons and Kaplan.[1] For conjugation, a 10% solution of egg albumen was prepared from fresh hen eggs and 1.0 mg. of fluorescein isocyanate, isomer 1 (weighed as amine), was used per 0.05 gm. of protein.

For removal of free-fluorescein derivatives, the method of ethyl acetate extraction, which had been used in most of the serum protein preparations,[2] was found to gel the conjugate; because of this, the original Coons-Kaplan technique [1] was employed. Free-fluorescein derivatives and excess dioxane and acetone were removed by dialysis at 1 C, with daily changes of dialyzate for five days, and single absorption with acetone-dried mouse-liver particles was performed.

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Unfixed frozen sections, taken from the margins of various tumors, were cut by the method of Louis [7] and stained for 15 minutes at room temperature with the conjugate. After washing for 15 minutes in three changes of phosphate-buffered saline, pH 7.3, the sections were examined, using a Leitz fluorescence microscope. Further technical details on staining with fluorescein-protein complexes, preparation of blood films, and ultraviolet microscopy and photography have already been described in detail.[7,8,11]

During the use of egg-albumen conjugates in the early stages of this study it was found that the sections lifted from the slides. This difficulty, however, was overcome by diluting the stain with 2 vol. of phosphate-buffered isotonic saline, pH 7.3.

Results

Sections prepared from various tissues, both innocent and malignant, taken from a series of animals were investigated after treating with fluorescein-labeled egg albumen, and the results were compared with those obtained after the same tissues were stained with other fluorescein-labeled serum protein fractions. Observations were made on the normal tissues of axolotl, mouse, rat, rabbit, dog, fowl, and man; on certain hyperplastic tissues; on embryonic tissues, and on a variety of malignant tissues. In each case identical staining affinities were observed irrespective of whether α-, β-, or γ-globulin, serum albumin, or egg albumen was incorporated in the fluorescein-protein complex which was used as a stain.

Normal Tissues.—Normal tissues examined routinely were liver, kidney, bowel, skin, blood, and brain. The cytoplasm of all epithelial cells showed a strong affinity for the conjugated dye and fluoresced brightly in ultraviolet light, whereas the connective tissue failed to do so. The red

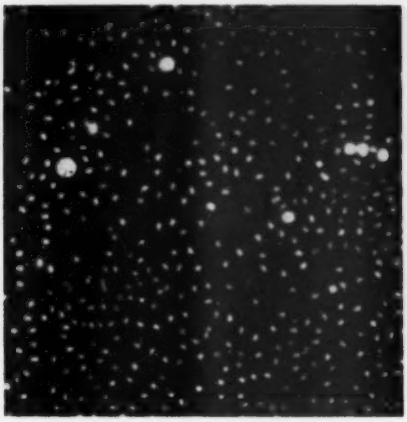

Fig. 1.—Fluorescence photomicrograph of blood film taken from a fowl and stained with fluorescein-egg albumen complex. Both white cells and nuclei of red cells fluoresce brightly. × 220.

blood cells also lacked this property, but in the lower vertebrates (birds, fish, and amphibia), in which the erythrocytes are nucleated, the nuclei of these cells did stain (Fig. 1). Brain tissue except for ependyma, on the other hand, from all the above-enumerated animals uniformly failed to show a positive staining reaction with any of the fluorescein-labeled protein complexes. Finally, the autofluorescence which was found to occur in keratin and elastic tissue was in no way altered or diminished by the egg-albumen conjugate.

Hyperplastic Tissues.—The staining reactions of both naturally occurring and induced hyperplastic states were investigated. In all these conditions found to occur in the breast (lobular hyperplasia, fibroadenoma), skin (warts, irritated epithelium), bowel (polyps), and thyroid (thyrotoxicosis, fetal adenoma) the tissues stained well (Figs. 2 and 3). This property was found also in the induced hyperplasias, such as regenerating rat liver (Fig. 4) and Shope papilloma of the rabbit's ear, which were studied in detail. Here all the actively growing liver cells, immediately following partial hepatectomy, stained just as normal liver cells. The proliferating epidermis of the Shope papilloma from the

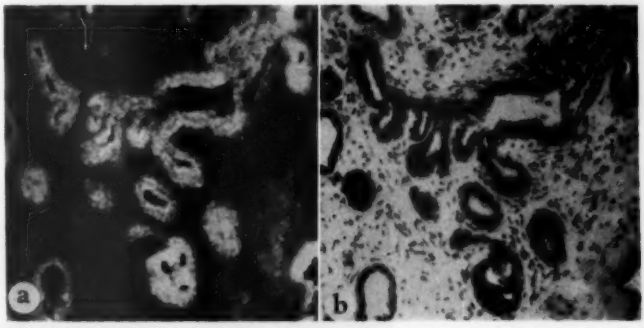

Fig. 2.—Lobular hyperplasia of breast (man): (*a*) Unfixed frozen section stained with fluorescein-egg albumen complex and showing uniform fluorescence of all epithelial cells lining the ducts; (*b*) same area after fixation in formalin and stained with hematoxylin and eosin for comparison with (*a*). Reduced 20% from mag. × 220.

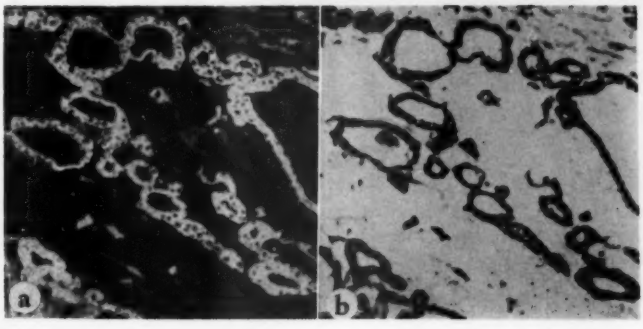

Fig. 3.—Fetal adenoma of thyroid (man): (*a*) Unfixed frozen section stained with fluorescein-egg albumen complex and showing bright fluorescence of all the small cuboidal cells. Neither intra- nor extra-acinar colloid stains. (*b*) Same area after fixation in formalin and stained with hematoxylin and eosin for comparison with (*a*). Reduced 20% from mag. × 220.

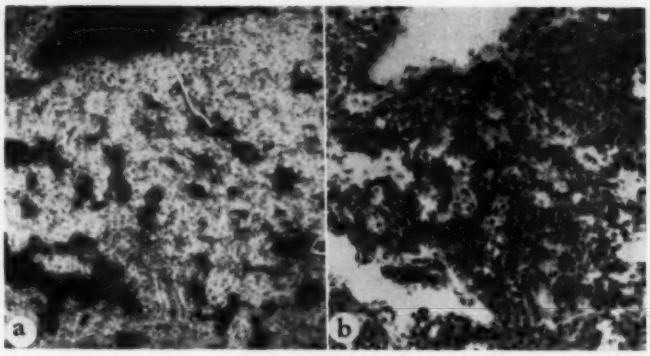

Fig. 4. — Regenerating rat liver 36 hours after partial hepatectomy: (*a*) Frozen, unfixed section stained with fluorescein-egg albumen complex and showing uniform fluorescence of cytoplasm of all parenchymal cells; (*b*) same area subsequently fixed in formalin and stained with hematoxylin and eosin for comparison with (*a*). Reduced 25% from mag. × 180.

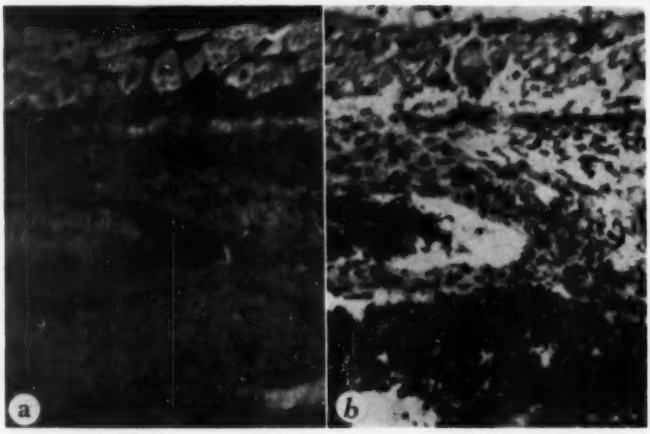

Fig. 5. — Transplanted Walker 256 breast carcinoma (rat): (*a*) Unfixed frozen section stained with fluorescein-egg albumen and showing a narrow strip of tissue fluorescing (on top); (*b*) same area subsequently fixed in formalin and stained with hematoxylin and eosin for comparison with (*a*). The fluorescing tissue is muscle, and the non-fluorescing area is the typical tumor tissue. Reduced 20% from mag. × 220.

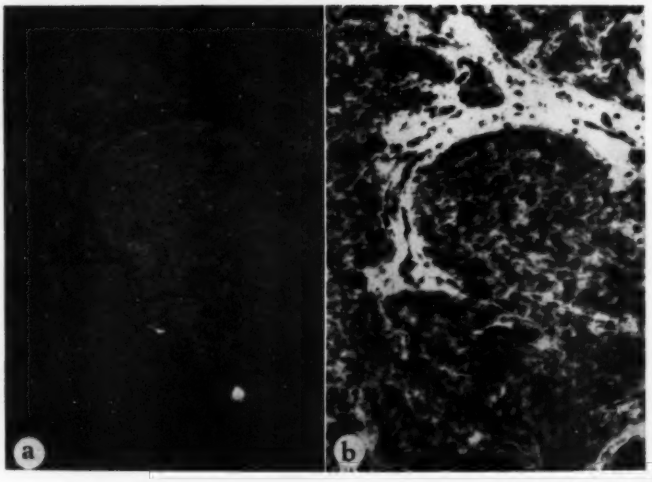

Fig. 6.—Carcinoma of cervix (man): (*a*) Unfixed frozen section stained with fluorescein-egg albumen complex and showing complete lack of fluorescence of all cells; (*b*) same area subsequently fixed in formalin and stained with hematoxylin and eosin to show the structure of the non-fluorescing tissue. Reduced 20% from mag. × 220.

initial stages until the ninth month of growth also fluoresced brightly.

Embryonic Tissues.—The tissues studied here were those of the bird (domestic hen), embryos of mouse and rat, embryos of man, and primitive marrow cells from man. In all these tissues the cells stained in a manner similar to that observed in the adult tissues. In the earlier stages of development of the chick embryo, before the formation of recognizable organs, the cells showed a bright fluorescence. This was seen also in the developing placenta and in the nuclei of the primitive red cells of the marrow (man). Subsequently a stain prepared from albumen of an incubated egg was shown to have an affinity for the tissues of the particular embryo of that egg.

Tumor Tissues.—All tumor tissues examined failed uniformly to stain with egg-albumen conjugate, as with other conjugated serum protein fractions (Figs. 5 and 6). Examples studied for this investigation were taken from the mouse (carcinoma of breast), rat (Walker 256 carcinoma), dog (carcinoma of breast), and man (carcinoma of lip, kidney, breast, colon, and prostate, and white blood cells from cases of acute leukemia).

Comment

This demonstration that various globulin and albumin conjugates gave differential staining identical with that ascribed by Weiler [12] to an organ-specific antigen-antibody reaction further demonstrates the immunologically nonspecific nature of the reaction. Such a finding is, however, fully in keeping with the view that the staining is dependent on differences in charge of proteins of the section and the stain, whereby salt-like complexes between proteins of opposite net charge are formed in staining. In these circumstances egg-albumen conjugates would be expected to be equally as efficacious as other albumin conjugates in producing differential staining—as indeed they have been found to be.

The differences in affinity of various tissues for fluorescein-protein conjugates have been found to be constant for the different types of tissue. Thus, normal and hyperplastic tissues have a strong affinity for the stain, whereas their neoplastic counterparts have lost this. That this is not simply a result of rapid cellular proliferation is shown by the findings that embryonic tissues stain well, as do those in active growth in the adult; cells in mitosis in regenerating rat liver stain well.[10]

There are some exceptions: Central nervous system does not stain, though the cells of the ependymal lining do; red blood corpuscles also fail to stain, although, where nucleated, as in birds, reptiles, amphibia, and fish, the nuclei stain well. The nuclei of the normoblast in man, in contradistinction to nuclei of other tissues, also stain.

Connective tissue does not stain, but this appears to be due to the predominance of intercellular substance, since, when connective tissue cells become large and contain a significant amount of cytoplasm, these cells also stain well. This applies especially to wandering cells, such as macrophages and plasma cells, and all white cells coming from the blood. The positive staining of swollen, fixed connective tissue cells is well demonstrated in inflammatory conditions and is easily demonstrated in foreign-body giant cells.

In addition to hyperplasias in experimentally induced regenerations, the tissues in various forms of naturally occurring hyperplasia, such as is commonly seen in the skin and the breast, also stain well. The epithelium in innocent tumors, such as the fibroadenoma of the breast, also fluoresces brightly, suggesting that, from the point of view of the features demonstrated by this method, they are essentially different from the malignant tumors.

All malignant tumors have failed to stain. This was first observed in the experimentally produced hepatoma of the rat and in the transplantable Walker 256 carcinoma

of the rat, but it has been observed also in naturally occurring tumors in animals, such as carcinoma of the breast of the mouse and the dog. It has also been noted in a wide range of naturally occurring tumors in man.

Examination of tissues from lesions generally considered to be preneoplastic, such as the early stages (before the development of the tumor) of amino-azo dye carcinogenesis of the liver in rats[3] and naturally occurring polyposis coli of man[9] show localized areas of cells which have lost their affinity for the stain. These areas, referred to as "islands of loss," have been considered to be neoplastic foci.[12]

Fluorescein-egg albumen conjugates prepared from incubated eggs have been found to stain sections of embryos of the donor eggs. This further supports the conclusion that the staining reaction is independent of any antibody-antigen reaction.

The consistent results obtained by this method indicate that it is of practical value in differentiating malignant from normal tissues in cases where morphological characters, using other methods, are too similar to allow easy differentiation.

Summary

A method for the preparation of fluorescein isocyanate-egg albumen conjugates is described, and the results of staining frozen sections prepared from the margins of tumors are described.

In common with many other conjugates prepared from different serum protein fractions from various animals, fluorescent egg albumen has been found to stain non-neoplastic, but not neoplastic, tissues.

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So-Called "Nuclear Pellets" ("Kernkugeln") of Pineocytes

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The concept embodying the term *Kernkugel* was formulated in 1901 by Dimitrova [1] from her research into the microscopic structure of the pineal parenchymal cell. Translated from German, the word means "nuclear pellet," or "nuclear sphere," the Greek equivalent of which would probably be karyo-+soma. The only extant research on the matter has been done in Europe and published for the most part in the German literature, thereby accounting for the perpetuation of the German word in pertinent, but scanty, non-German literature. Because foreign words are not universally understandable and require rote memory, it would seem more appropriate for our present purposes to discard *Kernkugel* in favor of its more meaningful translation, "nuclear pellet."

Subsequent studies led to a maze of controversies concerning the actual existence of such unique structures, their origin, age of occurrence, number, distribution among pineal cells and in glands of various species, their size, morphology, composition, and supposed function. A comprehensive review of the concept is necessary in order to unravel the manifold aspects of the controversies, as well as to implant them into the American literature.

Observations

Origin.—Among investigators who believe that the pellets constitute distinctly *de novo* structures peculiar to ontologically mature pineocytes, there are two schools of thought as to their ultimate genesis. The first, introduced by Dimitrova,[1] proposes that pellets arise intranuclearly but independent of nucleoli, a type of "essential" [our word] origin. Krabbe [2] and von Volkmann [3] adhered to this view, although they, too, could shed no additional light on the problem. On the basis of morphology and staining reactions, Josephy [4] tended to agree with this idea of an obscure intranuclear source. The second viewpoint was first propounded by Achúcarro and Sacristán,[5] who, from observations of morphology and composition, concluded that pellets represent intranuclear tube-like invaginations of cytoplasm which have been completely snipped off. Walter [6] agreed, stating that pellet contents considerably resemble the cytoplasm.

Meyer [7] was of the opinion that pellets are derived from alteration of nucleoli and are not primarily *de novo* structures. He described in elaborate detail the process whereby nucleoli apparently enlarge as vesicles by means of imbibition of fluid, become somewhat granular with a distinct enveloping membrane, and finally undergo degenerative homogenization with disappearance of granularity.

Age of Occurrence

There is almost unanimous agreement that pellets are not invariably to be found at any age. Equally unanimous is the opinion that their incidence increases with age.[3] Although in exceptionally rare (and probably pathological) instances they may occur in man even at the age of one year,[2] they first appear in significant numbers at approximately the end of the first decade of life, reach peak incidence at puberty, and remain diminishingly abundant during the productive period of life. Some authors

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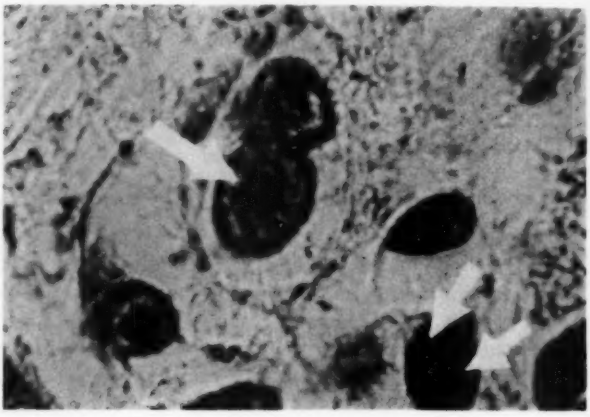

Fig. 1.—Several pineal nuclei bearing pellets, centrally located in largest nucleus (arrow at left center). Two dense pellets apparently occupy three-quarters of nucleus at lower right (lower two arrows). Human gland chrome alum-gallocyanin (cytoplasm not stained); approximately × 1,400.

stated that they continue to increase in number after puberty into old age; others adhered to the view stated above and were unable to detect many pellets after the age of 50.

Distribution in Glands and Species

Pineocytes bearing pellets are distributed irregularly throughout the gland,[3] being especially numerous in the peripheral fiber zone of each lobule.[3,8] They appear only in pineal parenchymal cells, although they may occasionally be seen in ependymal cells of the recessus pinealis during the first few years of life.[2] So long as the pellets are relatively small, they are usually located peripherally in the nucleus; otherwise, intranuclear position is random,[9] the largest usually being found in the center of chromatin-poor nuclei [4] (Figs. 1 and 2).

It is generally conceded that occurrence is rarer in animals other than man, but conflicting observations have been reported with regard to existence of pellets in other forms of life. One group of investigators stated that they are found in horses, cattle, sheep,[1] goats,[10] cats,[11] and white mice.[8] According to a solitary study,[12] they are also seen in rabbit glands, but this has not been confirmed. Bargmann [8] saw pellets in the pineal of one orangutang; but he, too, admits that primates have not been adequately studied in this regard.

On the other hand, dissenting viewpoints were published by Bargmann [8] and László,[13]

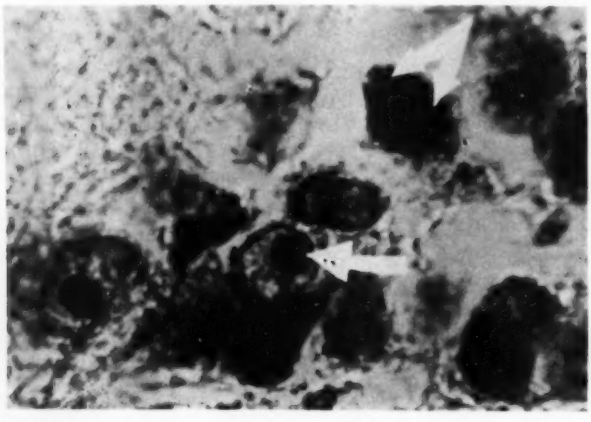

Fig. 2. — Numerous intranuclear pellets, a few apparently in process of "expulsion," especially that indicated by central arrow. Two large pellets in one nucleus at upper right (arrows). Pellet in nucleus at extreme left appears to have made contact with nuclear membrane. Human gland, approximately four hours after death. Gallocyanin; approximately × 1,400.

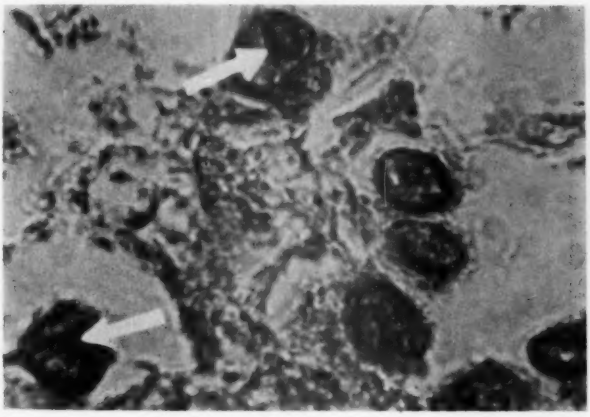

Fig. 3.—Chromatin deposits along perimeter of pellet (upper arrow) simulate an enveloping membrane. There is heavy accumulation of chromatin at tip of arrow (opposite point of contact of pellet with nuclear membrane). Note extreme nuclear folding at lower left (arrow). Adult human gland, several hours post mortem. Gallocyanin; approximately × 1,400.

who could find no pellets in cattle, sheep, and hogs.

It is noteworthy that pellets have even been seen in pinealomas of man [9,14] and horses.[13]

Number, Size, and Structure

When found, there is usually one pellet in each nucleus, but two or more per cell is a common finding [3,4,8] (Fig. 2). A single nucleus having five pellets has been seen.[3] Pellets average 4μ to 5μ in diameter [2,7,13,15] and are occasionally larger in older, chromatin-poor nuclei.[7]

The pellets may be round, bacilliform, or very irregular in shape. Each one may appear to be homogeneous and glassy,[1] or granular,[2,7,16] or a composite of peripheral hyaline laminations surrounding central granularity.[8] The last consideration is admittedly moot, however, since granularity may be nothing other than an artifact of alcohol fixation.[2,3] Granular pellets may occasionally contain dark-brown pigment.[7, 14,17] Although no enveloping membrane can definitely be discerned, according to most investigators, deposition of chromatin around the edge of the pellet can give such an impression [3] (Fig. 3).

Chemical and Staining Reactions.—The pellets do not stain for fat, glycogen, or iron.[4,7,15] Furthermore, they are not affected by distilled water, solutions of 1% and 10% NaCl, 2% $CuSO_4$, 1% and concentrated $MgSO_4$, 1% and stronger tartaric acid, isotonic NaOH, and 5% HCl.[4] Amyloid reactions are negative [15]; and, on the basis of a negative phosphoric acid reaction, Krabbe [2] concluded that pellets are not derivatives of phosphoric acid-containing nucleoli. In their homogeneous nature Polvani [15] saw a superficial resemblance to colloid of the thyroid and pituitary. Stains which have been used to demonstrate pellets are osmium tetroxide (black), Weigert's hematoxylin (gray), safranin (rose pink), Van Gieson (red-brown), "Azan" (blue), polychrome methylene blue, pyronin (red-pink), methyl green, light green, Feulgen method, carbol-fuchsin, Congo red, brilliant cresyl blue, and toluidine blue.

Postulated Significance.—An active, special cellular function has been ascribed to the pellets because their number and occurrence are independent of any retrogressive changes in the pineal parenchyma.[3,9] One group of workers [2,3,7,15] claims that the pellet and its contents ultimately pass from the nucleus into the cytoplasm. Although this has been referred to as "excretion" by some and as "secretion" by others, lack of agreement, together with lack of incontrovertible proof on this point, would favor the use of designations such as "extrusion" or "expulsion." Tissue culture work with living pineocytes may provide the answer.[8] The process [7] has been de-

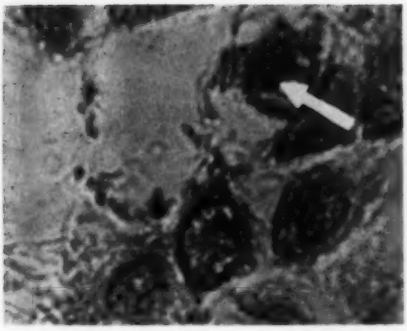

Fig. 4.—Nucleus in upper right may be construed to be in process of expelling pellet (arrow) into cytoplasm. Adult human gland. Gallocyanin; × 1,400.

scribed in great detail. As the pellet touches the nuclear membrane (Fig. 3), chromatin accumulates on its intranuclear surface opposite the point of contact. The nuclear membrane may rupture mechanically [11] or simply dissolve,[7] to release pyroninophilic pellet contents into adjacent cytoplasm. These "secretory products" are in general not as distinct as ordinary secretory granules. Because the point of contact may appear to bulge into cytoplasm before rupture, and also because the edges at the point of rupture (Fig. 4) are at first sharp and later become dull and round, and from computations of surface-tension coefficients, intracellular pressures, and radii of pellets, Meyer [7] inferred "expulsion" to be a karyofugal process—from nucleus into cytoplasm. Furthermore, he stated that if two pellets in a single nucleus were to make contact, their contents could be mutually exchanged in like manner even if they were in different stages of development. Also, one pellet (of the pair) may expel its contents into cytoplasm via another which is already in the process of doing so. When empty, the pellet shrinks to a tube, and then to a chromatin-laden rod or scar projecting into the nucleus from the nuclear membrane (Fig. 5), and finally disappears with restoration of nuclear continuity. Lobulation of pineal nuclei (Fig. 3), which increases with age, is said to be a direct consequence of alteration of nuclear volume through such expulsive action (which also tends to increase with age). Bargmann [8] felt that amitosis could in some measure account for the lobulation.

Contrariwise, Achúcarro and Sacristán [5] believed that pellets are a degenerative sign and compared pineal nuclear changes with age to the shrinking of nerve-cell nuclei. Walter [6] agreed with them as to the origin of pellets, inferring that the pyroninophilic granules at points of "expulsion" are artifactual and that the remarkable resemblance of the contents of rod-shaped pellets to cytoplasm is indicative of a karyopetal process—inclusion of cytoplasm within nuclei—and not vice versa. But he could not concur in the idea that pellets signify premature involution, for he saw them in

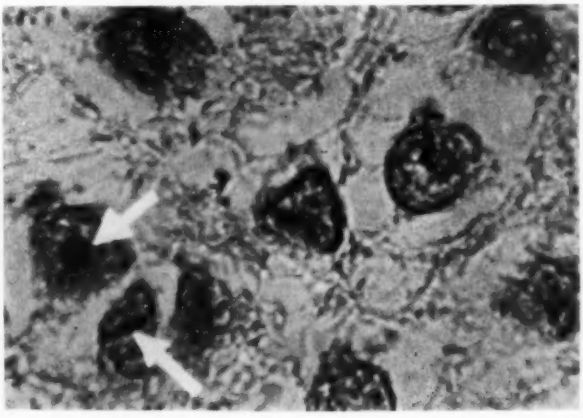

Fig. 5.—One nucleus at left has a round, homogeneous pellet (upper arrow); another has a linear, dense, rod-shaped pellet or "scar" (lower arrow). Adult human gland, several hours after death. Gallocyanin; approximately × 1,400.

hypertrophic (therefore not degenerative) cells, as well as in cells of the so-called "peripheral fiber zone" of glandular lobules, which, because of their numerous argyrophilic processes, have probably never had any secretory activity.

This is approximately the whole story up to the present time so far as pineal nuclear pellets are concerned—obviously an unsatisfactory sequence of controversial, albeit praiseworthy, deductions. With the advent of more refined tools and methods, it would seem worth while using them to attempt to bring some objective order out of the present subjective chaos. This is the fundamental aim of our investigation.

Materials and Methods

The most pressing problem is to determine whether the pellets (*Kernkugeln*) really are significant entities or simply misconstrued artifacts. With this in mind, a series of special stains was done on human pineal glands obtained at necropsy, half of each gland being fixed in alcohol and half in formalin, and routine paraffin-block sections of the two halves were submitted to the same stain simultaneously on the same slide. We repeated some of the stains used by previous authors in well-documented investigations in order to establish a base-line plane of reference. Other stains used have never before been reported in the study of pineal pellets. Glands were obtained from four women and seven men, the time after death ranging from 3 to 30 hours. In one case excessive lime salt deposits necessitated decalcification of the fixed specimen. The age range was from 36 to 79 years, a single patient being 15 months old. The observed results of these special stains examined under light microscopy (LMS) are summarized in the accompanying Table.

The electron microscope (EMS) seems to be the ideal tool for incontestable demonstration of pellets if they do indeed exist. Pineal glands from eight gray and five white adult rats and five white mice in apparent good health were removed within a minute after death, quickly sectioned in fine pieces, and immediately fixed in cold 1%-2% osmium tetroxide for an hour in the refrigerator. The fixed pieces were then dehydrated and blocked in methyl methacrylate plastic. Sections approximately 150-200 A. in thickness were cut on a mechanical ultramicrotome and examined in Zeiss and RCA machines. Autopsy material from two adult human glands (one and a half and four hours after death) was similarly handled; in one of these cases some bits of tissue were also fixed in 10% formalin at 0 C for two hours and then treated in cold 1% OsO_4 for an additional hour.

Besides the routine special stains referred to above, other preliminary studies using fluorescence microscopy (FMS), as well as enzyme and chemical digestion, were carried out in an attempt to elucidate a special nature and constitution of the pellets (assuming that they do exist). Glands from two adult, apparently healthy gray rats were placed in cold Carnoy's solution (absolute alcohol, chloroform, and glacial acetic acid, at 0 C) within a minute after death and allowed to undergo fixation overnight in the refrigerator. Also, each of three adult human glands was halved at necropsy

Results of Special Stains Under Light Microscopy

Stain	Formalin-Fixed Sections	Alcohol-Fixed Sections
Krause method (for colloid)	Chromatin light purple-gray; pellets, nuclear folds, nucleoli bright aqua-blue; occasional large, round, glassy pellet was purple-gray	Chromatin deep aquamarine-purple; pellets chiefly aquamarine, occasionally dark red-purplelike cytoplasm
Cresyl violet	Above structures deep violet; rather intense staining	Light blue-violet, less intense, more contrast; slight accentuation of violet in few scattered, large pellets
Feulgen (nuclear reaction)	Above structures bright purple; pellets more intense	All structures (nuclear) proportionately lighter and paler purple
Hemalum and eosin	Chromatin, nucleoli, pellets blue-gray, the latter more intensely	Same structures blue-purple
Congo red	Very light orange tinge on pellets and chromatin	Slightly darker orange; no differential staining
Giemsa (Pappenheim modification)	Above structures bright aquamarine	Nuclear structures smudgy gray-purple
Azan (Heidenhain)	Above structures light gray-orange	Very intense deep red-orange
Safranin	Above structures pink-red	Paler pink-red
Masson's trichrome	Above structures dark gray	Intense muddy brown
von Kossa	Negative	Negative
Masson's argentaffin method	Negative	Negative
Ziehl-Nielsen (acid-fast)	Negative	Negative
Periodic acid-Schiff	Negative	Negative

four to nine hours after death; one-half was fixed in Carnoy's solution, as above; the other, in cold formalin for the same length of time. All fixed material was subsequently blocked in paraffin and sectioned at approximately 7μ-8μ in thickness.

Several deparaffinized sections were examined by fluorescence unstained in each case. Others were stained with a 1:10,000 solution of acridine orange buffered to pH's 0.65 and 1.5 (Walpole buffer), 4.2 and 5.0 (McIlvaine buffer), and 6.4 and 8.0 (McIlvaine buffer) and examined by FMS. Furthermore, deparaffinized sections of one rat gland and one human gland of this series were immersed in solutions of ribonuclease (1 mg. of enzyme per cubic centimeter of tridistilled water in quartz cuvettes) and deoxyribonuclease (0.01 mg. of enzyme per cubic centimeter of tridistilled water) for periods of one, two, and four hours at 37 C. Control sections were placed in tridistilled water at the same temperature for corresponding lengths of time. Finally, deparaffinized sections of rat and human material were treated with 10% perchloric acid at 0 C for 18 hours, aimed at removing ribonucleic acid (RNA), and with 5% perchloric acid at 65 C for 20-45 minutes to remove deoxyribonucleic acid (DNA). Control sections were immersed in distilled water. All these sections treated with enzymes and chemicals, as well as their controls, were evaluated after staining for 12-18 hours with chrome alum-gallocyanin (a specific nuclear stain of the basic aniline-oxazine group).

Results

Scrutiny of LMS preparations revealed most of the findings described in the litera-

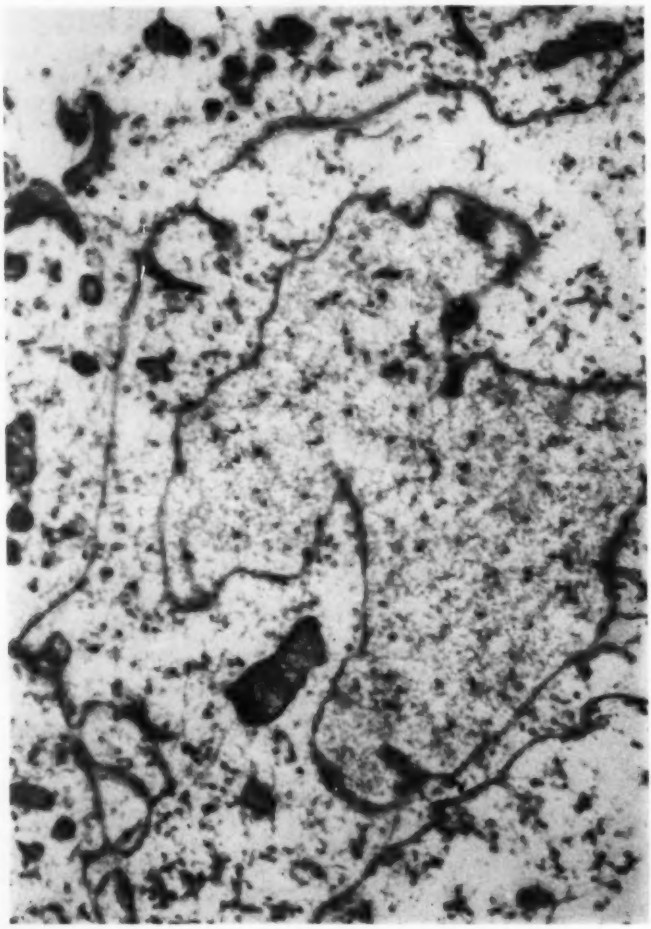

Fig. 6. — Pineal of white rat fixed within a minute after death. Note two large nuclear invaginations with a mitochondrion (dense rectangular body) contained in the lower one. EMS; approximately × 20,000.

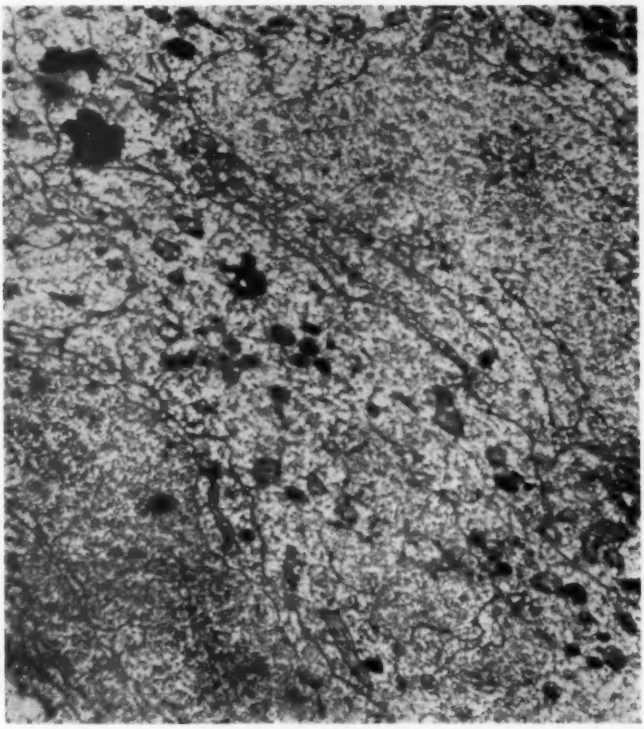

Fig. 7. — Excessively folded nuclei with many finger-like projections. EMS section of pineal of adult white rat. Gray structures are mitochondria; black spots are fat deposits. Reduced to 70% of mag. × 23,000.

ture. Pellets varied markedly in shape from perfectly circular to rod-like and extremely jagged; in size from slightly larger than nucleoli to those displacing three-quarters of the nuclear volume; in density from that of clear, hyaline vacuoles to the heavy-staining intensity of nucleoli. Generally abundance varied according to age, being greater in middle and old age. Few of the pellets seen in the infant's pineal body were discrete enough to be convincing. Pertinent results of this phase of the study are summarized in the Table.

The most striking nuclear finding under the electron microscope was shape: many nuclei were excessively folded and irregular

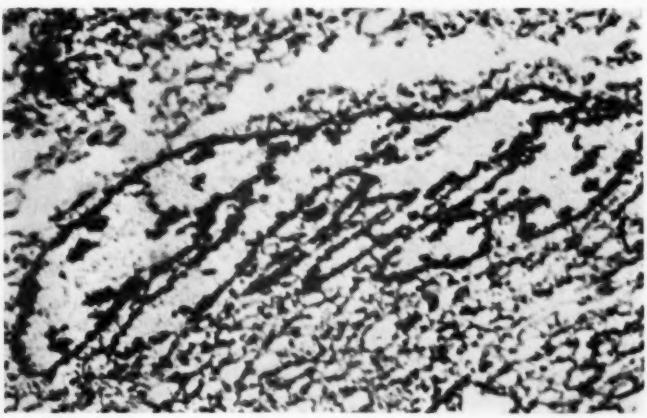

Fig. 8. — Adult human pineal gland, one and one-half hours after death. Numerous deep nuclear invaginations. EMS; reduced to 75% of approximate mag. × 10,500.

in outline (Figs. 6, 7, 8), in some cases actually looking like thick, coiled rope. It was not rare to observe multiple parallel finger-like projections of cytoplasm almost across the nucleus (Fig. 8). Special intranuclear bodies were not seen in the evenly dispersed chromatin. Occasional large nucleoli were easily discerned as such and were never confused with special bodies. Nucleoli sometimes exhibited internal, coiled, dense chromatin network, but never an enveloping membrane (Fig. 10). In human glands the five-hour postmortem specimen was so badly deteriorated structurally that EMS interpretation would have been meaningless in the discriminatory sense demanded by this problem.

Other irrelevant, but interesting, EMS findings were small, smoothly outlined foci of cytoplasmic lipidosis (black with OsO_4; Fig. 7) and small, irregular, intracellular calcific deposits, which also appeared dark and relatively homogeneous.

With FMS nothing was observed in unstained preparations, whereas in all specimens stained with acridine orange the chromatin and nucleoli, as well as "pellets" of human glands, fluoresced light yellow-green at pH 0.65 to 1.5, bright yellow at pH 4.2 to 5.0, and yellow-orange to bright orange at pH 6.4 to 8.0. The only variable noted from specimen to specimen was intensity of color, and in no case did a single nucleus show differences in quality of color among its constituents. The variable intensity was attributed simply to the differences in chromatin density of those structures (such as nucleoli).

Ribonuclease treatment of all sections was negative. However, gallocyanin staining of nuclei immersed in deoxyribonuclease solution was pale in comparison with controls, especially in the four-hour specimens. Intensity of the blue-black color in these pale nuclei corresponded exactly to the varying intensity of intranuclear structures, as noted above with stained fluorescence. In other words, there was no selective ferment action on the nucleus.

Results of perchloric-acid treatment were entirely similar to those of FMS. Nuclei in experimental and control sections subjected to 10% $HClO_4$ at 0 C for 18 hours stained exactly as did their controls. However, nuclei in sections treated with 5% $HClO_4$ for 45 minutes at 65 C were paler than their controls. All nuclear detail, including chromatin, nucleoli, and "pellets," was visible but proportionately obscured.

Comment

In the face of all reliable and convincing studies done earlier and our corroborative results with special stains, fluorescence, and enzyme and chemical studies, it was indeed a great disappointment not to have observed so-called nuclear pellets in pineocytes with electron microscopy. The latter tool must be considered the ultimate court of appeal at present. If its magnificent, shadowy penetration into the ultrastructure of fine cellular detail fails to reveal the existence of peculiar intranuclear bodies (known to be osmophilic) which are at all different from nucleoli or other established nuclear-structures, and which may occasionally be almost as large as the nuclei themselves, then on what justifiable grounds can such a concept be deemed valid? We can only infer that unique pellets per se probably do not exist. How, then, can we explain the undeniable presence of variable-appearing bodies in pineal nuclei with light microscopy—some clear and glassy, others staining similarly to, but more intensely than, surrounding chromatin?

Two basic phenomena, alone or in combination, can conceivably account for this paradox. Accordingly, pellets seen with light microscopy may be the result of (1) nuclear folds or (2) enlarged nucleoli or (3) a combination of the two.

In the first place, folds were noted even with LMS, using many different stains (Fig. 3). They were especially prominent, often to an extremely exaggerated degree, in EMS studies of human and rat glands (Figs. 7, 8). Overlapping of one or more

folds in relatively thick sections (8μ) probably accounts for the large, intensely staining, irregularly outlined "pellets" observed in LMS preparations, which often contain whole nuclei intact. Extra layers of chromatin along deep, horizontal, infolded corrugations of the nuclear membrane result in what appear to be variable-sized nuclear bodies. The folds can be fairly accurately traced by carefully focusing up and down with the high-power lens; and in some planes depth and degree of overlapping of folds produce unusual configurations, which can be easily interpreted as "expulsion" of bodies (Fig. 4). Since overlapping of well-outlined folds is impossible in the ultrathin EMS sections, large, intensely stained, variable-sized "pellets" could not be seen and most likely do not really exist.

Furthermore, certain cuts through corrugated nuclei, specifically, cuts perpendicular to the direction of folds, can produce the appearance of rod-shaped nuclear "inclusions." The contents of such "pellets" would of necessity resemble cytoplasm, because they would, in fact, be nothing other than intact cytoplasm projecting into a wide-based nuclear invagination. If the nucleus is not cut, and if a leaf-like invagination is in the direction of sight, the end-result will be a dense, rod-like (or ovoid and at times spherical) "pellet" with a very heavy outline. Only EMS sections are thin and clear enough to demonstrate this point. Ultramagnification also shows the "pellet" contents to be identical with the structure of surrounding cytoplasm (Fig. 9); mitochondria may even appear inside "pellets" (Fig. 6), as may lipochrome granules also. Thus, the concept of Achúcarro and Sacristán[6] is in part correct, but only to the extent that cytoplasm *appears* to have been incorporated into the nucleus.

Slightly enlarged nucleoli offered the sole structural evidence of definite intranuclear "bodies" with the electron microscope (Fig. 10). Their ultrastructure consisted of fine, matted, densely osmophilic filaments of chromatin, as already described in neurons.[18] Enlargement was not a common finding in our study. In some instances central density was less marked, and this finding probably accounts for the clear, vacuolar type of "pellet" seen with LMS. A distinct perinucleolar membrane was never seen in either human or rat specimens. Therefore it appears that there is some degree of plausibility in Meyer's original idea that pellets are altered nucleoli.[7]

Attribution of recently acquired knowledge about neuronal nucleoli to nucleoli of pineocytes further supports the above conclusion. It is known that nucleoli of human

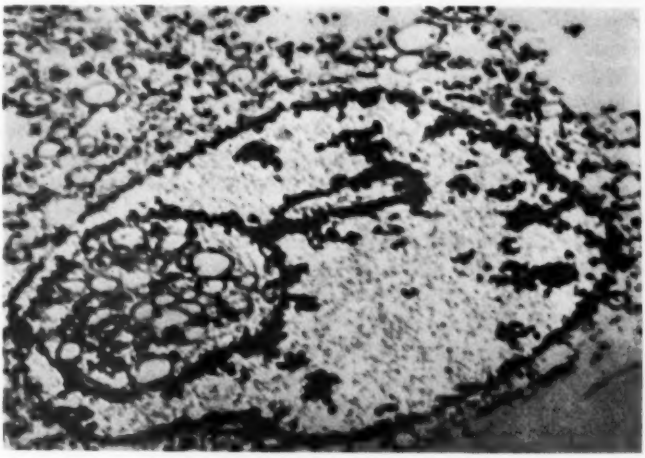

Fig. 9. — Adult human gland approximately one and one-half hours post mortem. Two nuclear invaginations so cut as to resemble spherical and bacilliform nuclear "bodies." Cytoplasmic nature of the "bodies" is obvious. EMS; reduced to 84% of approximate mag. × 10,500.

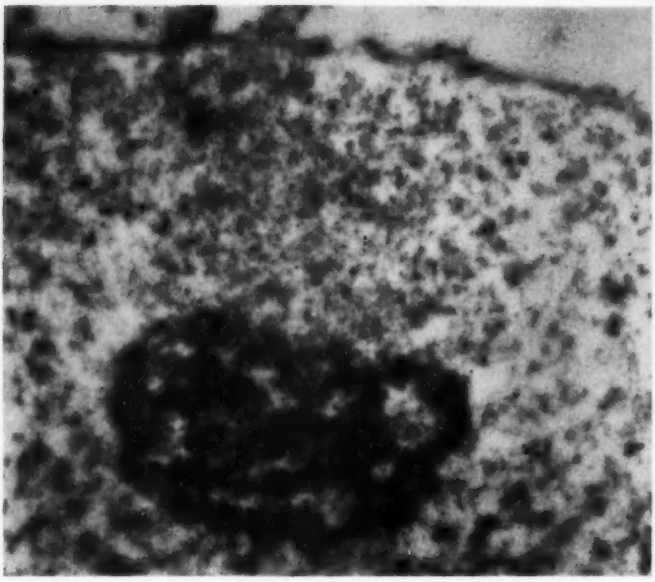

Fig. 10. — Slightly enlarged nucleolus of mouse pineocyte. EMS; reduced to 92% of approximate mag. × 35,000.

neurons are composed of Feulgen-positive nucleotides [19] and are commonly seen along the nuclear membrane, perhaps even jutting out like a knob into adjacent cytoplasm.[19] One or more tiny vacuoles may regularly be observed in each nucleolus,[20] but their significance is a disputed point. Formation of vacuoles in nucleoli of neurons increases with every temporary enlargement of the nucleus, a fact which has been emphasized as indicative of a peculiar metabolic or secretory activity of nucleoli.[21,22] In addition, other authors claim to have detected definite signs of nucleolar secretion [23] which gives rise to changes in nuclear form.[24] Recent phase-contrast studies of various types of living tissue-culture cells have corroborated these claims.[25] The description of the process of secretion of stored nucleolar contents [24] (said to be special chromosomal products) in neurons closely parallels that of "expulsion" of pellets as described by Meyer,[7] even to the observation of an ensuing rent surrounded by chromatin in the now-folded nuclear membrane.[26] Finally, histochemical proof of a specific pineal secretory function of unknown significance in rats has been obtained by Quay,[27] who noted that stained secretory products first appear in that portion of the cytoplasm adjacent to the segment of the nuclear membrane toward which enlarged nucleoli have previously shifted.

Size of "pellets" derived from nucleoli is probably directly proportional to the length of time between death of tissue and fixation. None of the nucleoli of rat and mouse glands studied under EMS and FMS were remarkably enlarged. In these cases fixation was necessarily very prompt. In two instances fixation of rat glands was delayed at least an hour (exact time unknown), and hemalum and eosin stains revealed many large, clear, vacuolar "pellets," similar in all respects to those seen in human autopsy material. This finding is undoubtedly the manifestation of postmortem degeneration. Many densely stained folded-type "pellets" were also noted.

Another disappointment was our failure to demonstrate significant differential staining of "pellets." Krabbe [2] claimed that in alcohol-fixed glands the bodies stain red with methyl green and pyronin, whereas chromatin itself stains green. Nucleic acids of nerve cells have been divided into two

types on the basis of histochemical reactions: (1) those having RNA content (red with methyl green and pyronin) and (2) those with DNA (green with the same stain).[26] Krabbe's results would indicate a preponderance of RNA in "pellets," in reality being RNA in the cytoplasm of nuclear invaginations. Nevertheless, in our results "pellets" stained qualitatively like chromatin, that is, green with the above-mentioned reagents. This observation in conjunction with enzyme and chemical digestions and fluorescence studies compels us to contradict him with the conclusion that when they are present, and whatever they are, "pellets" consist chiefly, if not entirely, of DNA. The only inconsequential hints of differential staining were noted in alcohol-fixed material stained with cresyl violet and in formalin-fixed sections subjected to the Krause method for colloid (Table).

In conclusion, several valid inferences can be drawn from the foregoing considerations. 1. Discrete intranuclear bodies, with or without special secretory or similar metabolic activity, especially characteristic of pineocytes, in all likelihood do not exist. Therefore the term "nuclear pellet" (*Kernkugel*) is misleading and unjustified. 2. Any nuclear body observed in living or in instantaneously fixed cells would probably be an enlarged nucleolus undergoing physiological changes in volume, associated with a known, but obscure, secretory process; and any additional name for such a structure other than "nucleolus" (or perhaps "macronucleolus" merely to distinguish its size) would serve no conceivably useful purpose. 3. The large vesicular pineal nucleoli which appear when fixation is delayed could likewise best be called "macronucleoli," or even "necronucleoli," to account for the cause and degenerative nature of their striking appearance. 4. Finally, since the multifarious shapes, sizes, and densities created by various degrees of nuclear folding and overlapping are artifacts in the purest sense, it would seem logical to refer to these pellets as "false nuclear bodies" or "false pellets" ("pseudokaryosomes," *"pseudo-kernkugeln," "falsche Kernkugeln"*) to emphasize that point. Such new technical terms themselves are unimportant; they can be of value only insofar as they serve as shorthand means of bearing these various points in mind and of facilitating accurate evaluation in the light of present knowledge of the enduring enigma that is the pineal nucleus.

Summary

According to a concept originated and developed solely by European investigators during the last half-century, there are unique intranuclear bodies (*kernkugeln* or "nuclear pellets") in pineocytes of various mammals (including man) which ostensibly increase in number with age and which tend to be extruded periodically into cytoplasm —a phenomenon of unknown significance. The present study involved many special stains under light microscopy (LMS), fluorescence microscopy (FMS), nuclear digestion with enzymes (for RNA and DNA) and perchloric acid, and electron microscopy (EMS) of human, rat, and mouse glands in order to demonstrate conclusively whether or not such "pellets" exist, and, if they do, to gain some insight into their specific composition. LMS could do no more than corroborate the work of previous investigators. FMS and digestion studies gave no additional data, failing to show any characteristic difference between "pellets" and other nuclear structures. EMS consistently failed to substantiate the apparent presence of "pellets" with LMS techniques and therefore justified the inference that "pellets" peculiar to pineocyte nuclei do not in fact exist as such; that they are in all likelihood simply misconstrued artifacts in relatively thick LMS preparations represented by overlapping and unusual views of nuclear folds, lobulations, and invaginations, and that the only real "pellets" can be nothing other than enlarged nucleoli which are undergoing either a normal physiologic process of intranuclear

secretion or a retrogressive phase of necrosis.

We wish to express our sincere gratitude to Prof. Herwig Hamperl for placing at our disposal the facilities of the Pathologisches Institut, Venusberg, Bonn, as well as the services of his skilled technicians, Misses Haland, Eder, and Kranefuss. We should also like to thank Dr. Norman E. Kemp, of the Department of Zoology, University of Michigan, for his kind assistance.

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Pathologisches Institut, Venusberg, Bonn, Germany (Dr. Wessel).

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Occlusive Disease of the Abdominal Aorta Associated with Panarteritis

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The clinical manifestations and treatment of aorta-iliac thrombosis have been adequately described since Leriche [1] first recognized the syndrome, in 1923. Owing to the fact that the disease mainly affects men in their 40's, the pathological picture is usually complicated by atherosclerosis, and the latter condition has been accepted by most people as being the underlying pathological process. Following the advances in vascular surgery, more material has become available for detailed histological examination, and recently Halpert and associates [2] have focused attention on the inflammatory process which is so frequently associated with this condition.

The purpose of the present paper is to describe three cases of occlusive disease of the abdominal aorta occurring in South African Bantu. In these people atherosclerosis is distinctly of a lower degree of severity, and they, therefore, provide less complicated material for the study of the primary disease process. Furthermore, these cases were of varying clinical duration, thus enabling us to study different phases of the disease.

Report of Cases

CASE 1.—A Bantu man, F. M., aged approximately 40, was admitted with a history of symptoms of three days' duration. He said that initially both his legs had been swollen but that subsequently the swelling on the right side diminished. The left leg remained painful, and he was unable to stand on that side.

Examination revealed that he was sparely built and that the mucous membranes were pale. His blood pressure was 100/60 mm. Hg., and his pulse rate was 100 a minute. Apart from a slightly tender prostate gland, general examination revealed no other significant findings. The left foot was swollen; the left leg below the knee was cold, and the muscles were tender. He could not perform active movements, and passive movements caused pain. The femoral, popliteal, posterior tibial, and dorsalis pedis on the left side and the posterior tibial pulses on the right side could not be felt. A translumbar aortogram done on the day after admission demonstrated complete occlusion of the left common iliac artery.

At operation on the same day, the left external iliac artery was found to be occluded by old and recent thrombus, and thrombectomy was performed.

The following day the patient died, unexpectedly.

Autopsy Findings

Apart from severe edema of both lungs, the significant gross pathological findings were confined to the cardiovascular system. The heart weighed 270 gm. and was macroscopically normal. The aorta showed minimal fatty streaking of the thoracic and abdominal portions. The common iliac artery on the left side was completely occluded by a 1½ in. long, recent thrombus, which came to within an inch of the bifurcation. This thrombus was of recent origin and had probably developed postoperatively. The internal iliac artery on the same side was occluded by a much older, pale thrombus. No macroscopic cause for the thrombosis could be detected.

Histological Examination

Sections were taken from most of the internal organs, and, apart from a lower-nephron nephrosis and hemosiderosis of the liver and spleen, nothing of significance was noted. The blood vessels in these organs were normal.

Vascular System

Blocks were taken from the femoral and the internal, external, and common iliac arteries on the left side, and from the abdominal aorta, thoracic aorta, pulmonary artery, brachial arteries, and common carotid arteries. Sections from each of these blocks were stained with hematoxylin and eosin, and the Masson-Verhoeff and toluidine blue

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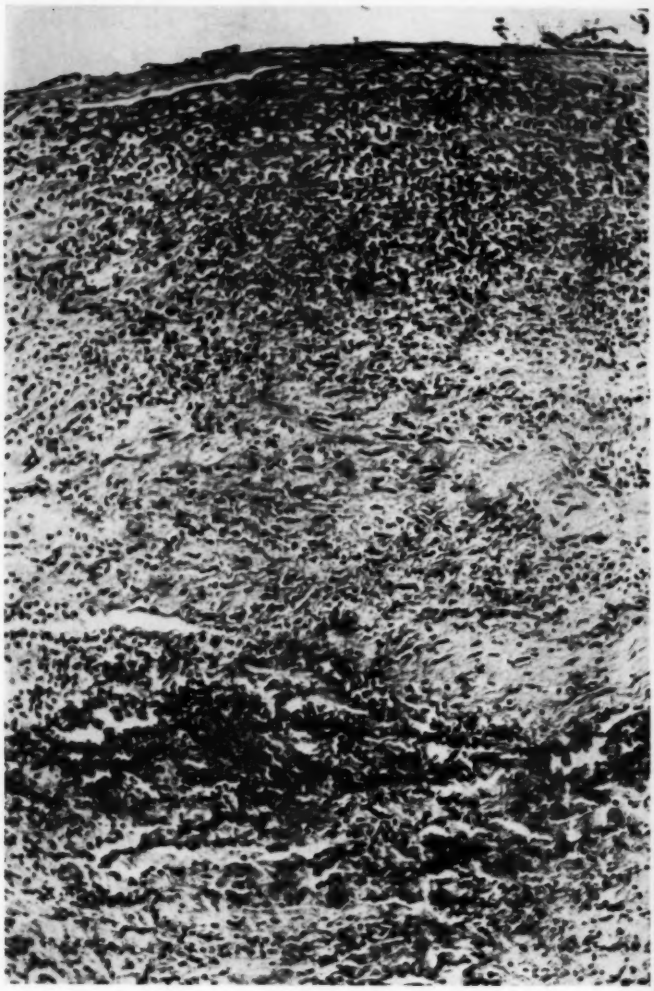


Fig. 1 (Case 1).—Section of the common iliac artery, showing acute panarteritis. Hematoxylin and eosin; × 80.

methods. The pulmonary artery, thoracic aorta, common carotid, and brachial arteries were normal. The femoral artery showed early medial calcification. The abdominal aorta showed a patchy lymphocytic infiltration around the vasa vasorum. In the common, internal, and external iliac arteries, however, there was a marked acute panarteritis (Figs. 1 and 2), with patchy degeneration of the elastic tissue and pyknosis of muscle nuclei. The intima of these vessels was covered with fibrin thrombus (Fig. 2), and only in the common iliac artery was there evidence of slight fibrous thickening of this layer.

Cause of Death

Postoperative shock with lower-nephron nephrosis.

Case 2.—J. K., a Bantu man aged approximately 60, gave a three weeks' history of swelling of the legs, chest pain, cough, and hemoptysis. The chest pain was stabbing in nature and worse on the right side.

Examination showed a poorly nourished, dehydrated, thin, elderly man, who appeared to be acutely ill. The blood pressure was 100/80 mm. Hg. The jugular venous pressure was slightly raised,

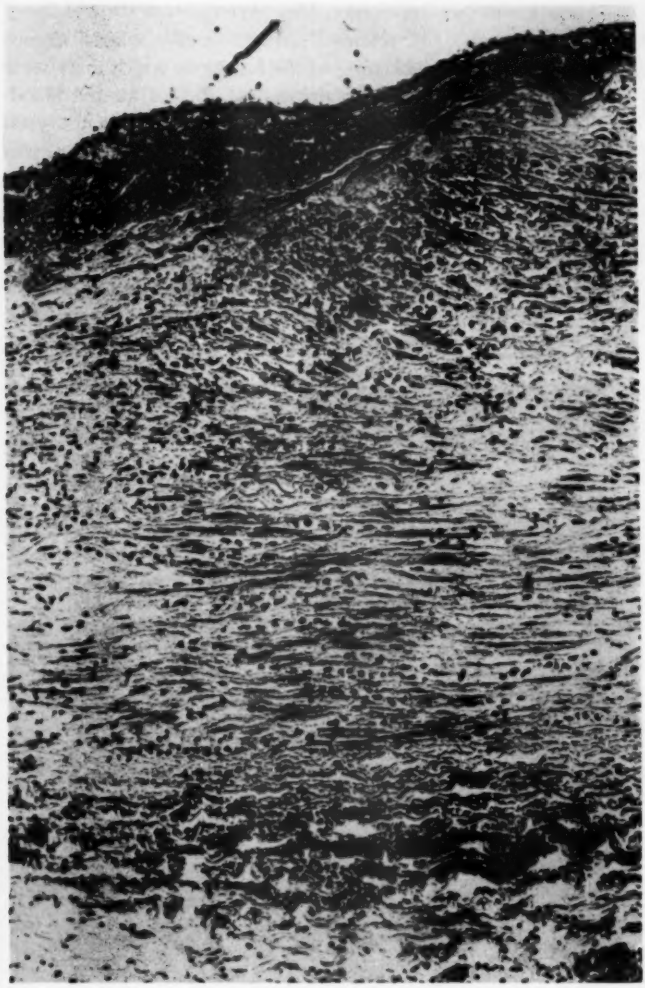


Fig. 2 (Case 1).—Section of the left internal iliac artery with acute panarteritis and early intimal thrombus formation. Hematoxylin and eosin; × 80.

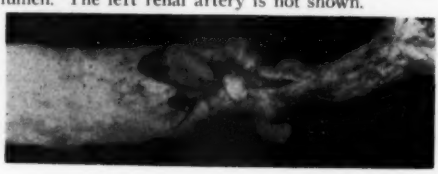
and there were tender hepatomegaly and ankle edema. Crepitations were present in the bases of both lungs. The heart was clinically enlarged. No pulses were palpable in the legs.

In spite of treatment, the patient died on the second hospital day.

Autopsy Findings

Confluent bronchopneumonia was present in the lower lobes of both lungs. The heart weighed 420 gm. and was enlarged, owing to left ventricular hypertrophy. The thoracic aorta showed mild to moderate atherosclerosis. The abdominal aorta was markedly narrowed and in segments, being totally occluded below the level of the renal arteries. This change extended into both common iliac arteries. There was also marked narrowing of the left renal artery, and the lumen of the aorta for a short distance above the renal arteries was partially occluded by a recent antemortem thrombus (Fig. 3). As a consequence of the nar-

Fig. 3. (Case 2).—Lower thoracic and abdominal aorta. The abdominal aorta is markedly narrowed up to the level of the renal arteries, and above this a recent thrombus projects into the lumen. The left renal artery is not shown.



rowed renal artery, the left kidney was atrophic. A bilharzic cystitis was present.

Histological Examination

Sections of the internal organs confirmed the presence of confluent bronchopneumonia, bilharzic cystitis, and nephrosclerotic changes in both kidneys.

Blocks were taken from different levels of the abdominal aorta and the common iliac artery and stained with the techniques mentioned in Case 1.

Common Iliac Artery

Sections showed well-marked fibrous thickening of the intima, in which there were areas of calcification, foci of altered blood-pigment deposition, and fatty-acid-crystal formation. The lumen of the vessel was severely narrowed, owing to intimal thickening and, furthermore, was almost completely occluded by a recent antemortem fibrin thrombus. The media was much thinner than normal and showed neovascularization and a patchy perivascular lymphocytic infiltrate (Fig. 4).

Fig. 4 (Case 2).—Section of the common iliac artery, showing fibrous intimal thickening and fatty-acid-crystal deposition, but without evidence of an arteritis. Hematoxylin and eosin; × 80.

Abdominal Aorta

Sections of the lower part of the abdominal aorta showed a histological picture essentially similar to that described in the common iliac artery.

Sections taken at the level of the renal arteries, however, showed much less, but still diffuse, fibrous thickening of the intima. The latter layer was covered by a very recent antemortem thrombus. The most striking changes were present in the media, where there was a diffuse infiltrate, consisting of lymphocytes, polymorphonuclear leukocytes, histiocytes, and occasional plasma cells (Fig. 5). Neovascularization in this part of the vessel wall was also fairly prominent. A similar infiltrate was present around the vasa vasorum.

Cause of Death

Confluent bronchopneumonia.

CASE 3.—J. M., a 30-year-old Bantu man, was admitted to hospital with a history of having had his left lower leg amputated for gangrene of the foot. While waiting for the prosthesis, a painful ulcer started to develop on the right foot. The entire right leg felt cold, and no pulses could be detected. The blood pressure was 120/70 Hg.

Fig. 5 (Case 2).—Section of the aorta at the level of the renal arteries, with a marked pleomorphic inflammatory-cell infiltrate of the media and adventitia. Hematoxylin and eosin; ×80.

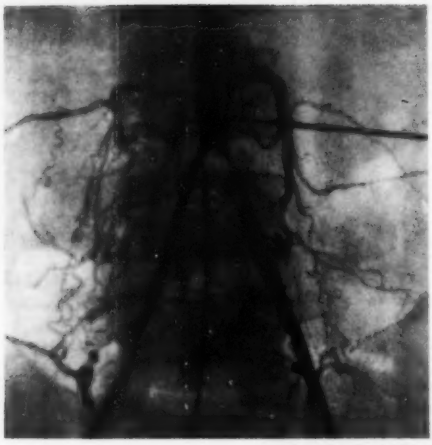


Fig. 6 (Case 3).—Aortogram showing narrowing of the left common iliac artery at its origin and narrowing of the right common iliac artery just above the sacrum. The collateral circulation is well demonstrated.

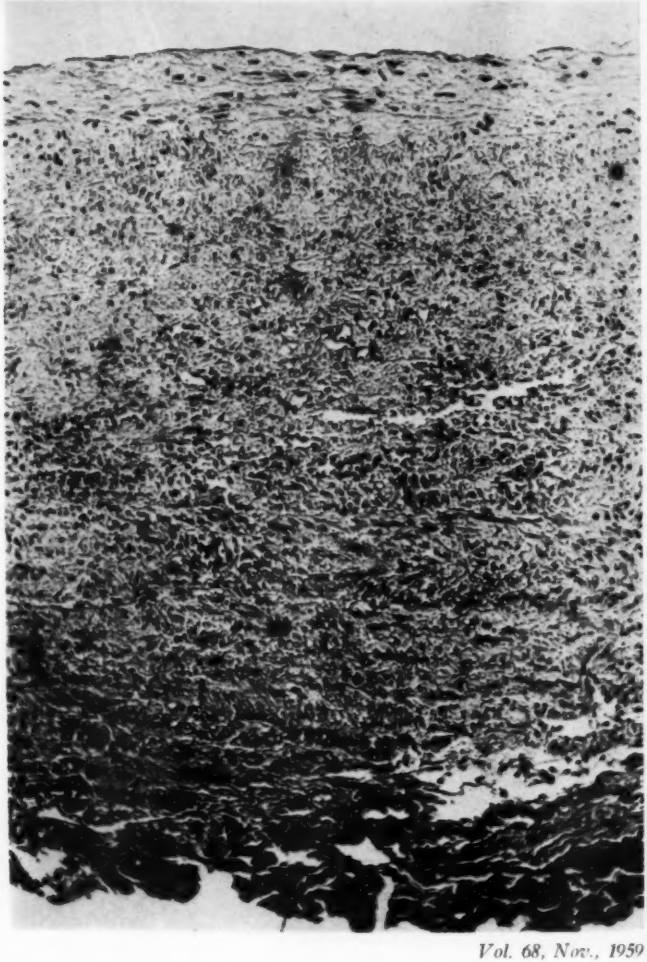


Fig. 7 (Case 3).—Section of the aortic biopsy specimen, showing the inflammatory-cell infiltrate in the media. Hematoxylin and eosin; × 80.

An aortogram showed narrowing of the left common iliac artery at its origin. On the right side, the common iliac artery was narrowed just above the sacrum (Fig. 6). The distal vessels of the right leg were well demonstrated and appeared normal.

At operation a "bypass" Dacron prosthesis was fitted from the bifurcation of the aorta to the femoral artery 1 in. above the origin of the profunda femoris. A biopsy specimen from the aortic wall was taken at the same time.

The postoperative course was uneventful.

Histological Examination

Sections of the aorta showed well-marked fibrous thickening of the intima. The media was the seat of widespread infiltration by lymphocytes, plasma cells, and occasional polymorphonuclear leukocytes (Fig. 7). There was also evidence of neovascularization, small foci of hemorrhage, and irregular areas of necrosis of muscle fibers. The adventitia showed a scanty perivascular lymphocytic infiltrate distributed around the vasa vasorum.

Comment

In reviewing the literature on the etiological aspects of primary occlusion of the abdominal aorta, it becomes apparent that atherosclerosis has been accepted as by far the commonest underlying cause of the thrombosis. This concept has been carried forward in spite of inadequate morphological studies on most of the original material. Occasional reference is made to other causes of primary occlusion in adults, such as dissecting aneurysm of the aorta, syphilitic aneurysm, trauma of the lower abdomen, neoplasms of the lumbar vertebrae, uterine neoplasms, and thromboangiitis obliterans.

Recently, however, Halpert and associates [2] have reported their morphological observations on 45 abdominal aortas which had been resected because of occlusive disease. In addition to the atherosclerotic and calcific lesions, they observed a "constant focal or diffuse chronic inflammatory reaction," and they, therefore, suggest that the inflammatory process may play a part in precipitating the condition. They also point out that the syndrome may be found in the presence of insignificant atherosclerotic changes elsewhere. Boyd,[3] although accepting atherosclerosis as the underlying factor, points out that the histological picture presents evidence of three entities, viz., periarteritis, medial calcification, and medionecrosis. These observations, then, certainly throw doubt upon the accepted etiological role of atherosclerosis in this condition.

In the South African Bantu it has been conclusively proved that atherosclerosis is of a much less degree of severity,[4,5] and that microscopically relatively normal aortas may be found until a fairly late age.[6]

Apart from the fact that not one of our patients had severe atherosclerosis, we have had the opportunity of studying one case (Case 1) in the acute phase in which undoubted evidence of extensive acute panarteritis was obtained in the common iliac and internal and external iliac arteries. The abdominal aorta at this stage showed only a mild inflammatory reaction in the adventitia. In one of the other cases (Case 2) the disease picture at or near the bifurcation corresponded to that of most of the chronic cases described in the literature. In the vicinity of the renal arteries, however, there was still evidence of an active subacute arteritis. It is quite conceivable that, if only the occluded part of the vessel were examined, the condition could have been ascribed to atherosclerosis alone. This observation may be of significance in that the disease appears to remain active and progressive for a long time in some cases.

We consider that these findings may in part explain the clinical impression of the relatively high frequency of peripheral vascular lesions in the Bantu,[7] in spite of the undoubted rarity of occlusive coronary artery disease.

Summary

The morphological changes occurring in three cases of occlusive disease of the abdominal aorta in the South African Bantu are described.

Evidence of active panarteritis was found in all three cases. It is suggested that the inflammatory reaction was the primary cause of the condition in these cases.

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On Aortic and Coronary Atherosclerosis in Cholesterol-Fed Cockerels

Histochemical Effects of Estrogens

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In previous studies,[1-6] it was demonstrated that estrogens prophylactically inhibited and therapeutically reversed coronary atherosclerotic lesions in cholesterol-fed cockerels. These effects of estrogens supervened in the apparent absence of any influences on aorta atherogenesis; i.e., definite segmental differences in atherogenesis were noted between the coronary arteries and the aorta.

Based on these findings, the present histochemical study was undertaken with the following objectives: (*a*) to reevaluate whether or not lipid deposition precedes other changes in intimal architecture in the process of atherogenesis; (*b*) to ascertain whether refined histochemical methods reveal any basis for the difference in atherogenic responses of aorta and coronary arteries to cholesterol plus estrogens; (*c*) to determine the finer architectural alterations, if any, in the coronary arteries of cholesterol-fed chicks protected against coronary atherogenesis by estrogens; (*d*) to analyze histochemically the residual pathologic alterations, if any, in the coronary arteries of chicks exhibiting estrogen-induced reversal of coronary atherosclerotic lesions.

Material and Methods

The special histologic and histochemical studies presented in this report were carried out on material from experiments with estrogens in cholesterol-oil-fed cockerels, previously described.[1,2] The special staining techniques were accomplished in the Buenos Aires laboratory, utilizing formalin-fixed hearts and their corresponding aortas from four groups of five animals each, forwarded by messenger as unknowns from the Chicago laboratory. Only after the findings on individual specimens were forwarded to Chicago were the data correlated according to the several experimental groups involved.

The four groups used were made up as follows: Group 1 was fed commercial Chick Starter Mash, supplemented with 2% cholesterol and 5% cottonseed oil (2 C-O) for 12 weeks. Group 2 received the same diet plus daily intramuscular injections of 1 mg. estradiol benzoate U.S.P. in oil.[1]* Group 3 and 4 received the 2 C-O diet for 13 weeks; in addition, Group 4 received daily injections of estrogens (1 mg.) for the last 5 weeks of the experimental period.[2]

In each specimen, the aortas were divided into three segments, superior, middle, and inferior, with the exception of those in Group 2, which were divided into two segments, a superior and an inferior. Each segment was studied grossly with a magnifying lens. For microscopic examination, selection was made of the severest lesion, as

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Now at the Instituto de Fisiología, Facultad de Ciencias Médicas, Universidad de Buenos Aires (Dr. Malinow). Established Investigator, American Heart Association (Dr. Pick). Work done while an Established Investigator of the American Heart Association; now Director of the Heart Disease Control Program, Chicago Board of Health (Dr. Stamler).

* We are indebted to Dr. Edward Henderson, of the Schering Corporation, Bloomfield, N.J., for generous supplies of estradiol benzoate (Progynon Benzoate).

TABLE 1.—*Microscopic Histochemical Gradings of Intimal Abnormalities in Sections of Grossly Normal Aortae Exhibiting Cholesterol-Lipid Deposition Plus Other Histochemical Changes*

Group	No. of Microscopically Abnormal Sections	Basophilic Cells	Foam Cells	Macrophages	Reticulum	Elastic Tissue	Collagen	Cholesterol *	Cholesterol Esters	Calcium	Metachromasia	Glucoprotein	Mucopolysaccharides	Hyaluronidase
1		2 †	4	1	2	0	3	5	4	0	3	3	3	3
2 C-O ‡	5	0.8 §	1.0	0.2	0.8	0.0	0.6	2.6	1.4	0.0	0.8	1.2	1.2	1.0
2		2	3	2	1	0	0	3	2	0	3	1	1	1
2 C-O+estrogen prophylactic	3	1.3	1.0	0.7	0.7	0.0	0.0	2.7	1.0	0.0	1.7	0.7	0.7	0.7
3		0	0	--	0	--	0	--	--	0	--	--	--	--
2 C-O	2	0.0	0.0	--	0.0	--	0.0	2.5	2.5	0.0	0.5	1.5	--	--
4		--	--	--	--	--	--	--	--	0	0	--	--	--
2 C-O+estrogen therapeutic	3	2.0	0.3	--	1.0	--	1.0	2.0	2.0	0.0	0.0	1.3	--	--

* Based on the Lieberman-Burchardt reaction.
† Number of sections with this finding in intima.
‡ 2 C-O is 2% cholesterol plus 5% cottonseed oil.
§ Mean of gradings, including sections graded 0.

TABLE 2.—*Microscopic Histochemical Gradings of Intimal Abnormalities in Sections of Grossly Detectable Aortic Lesions*

Group	No. of Sections	Basophilic Cells	Foam Cells	Macrophages	Reticulum	Elastic	Collagen	Cholesterol	Cholesterol Esters	Calcium	Metachromasia	Glucoprotein	Mucopolysaccharides	Hyaluronidase
1		8*	8	5	8	0	9	9†	9	2	9	9	9	9
2 C-O‡	9	1.7§	1.2	0.9	2.3	0.0	1.7	2.9‖	1.7	0.2	2.0	2.4	2.2	1.8
2		6	6	6	6	0	4	6	2	1	5	6	6	6
2 C-O+estrogen prophylactic	6	2.3	1.7	2.0	2.5	0.0	0.8	3.0	0.5	0.2	1.8	2.8	2.5	1.8
3		--	--	--	--	--	--	--	--	--	--	--	--	--
2 C-O	10	0.2	2.0	--	1.9	--	1.8	2.8	2.5	0.2	1.2	1.8	--	--
4		--	--	--	--	--	--	--	--	--	--	--	--	--
2 C-O+estrogen therapeutic	9	2.1	1.0	--	2.2	--	1.3	3.0	3.0	0.0	1.0	2.0	--	--

* Number of sections with this finding in intima.
† Windaus reaction.
‡ 2 C-O is 2% cholesterol plus 5% cottonseed oil.
§ Mean of gradings, including sections graded 0.
‖ Lieberman-Burchardt reaction.

judged by area of involvement and degree of elevation. When no gross lesion was found, the central part of the segment was selected.

Frozen sections, 8μ thick, were prepared, and each of the following techniques was used on three or four consecutive sections: (1) hematoxylin and eosin; (2) Gallego for elastic fibers; (3) Sudan IV for fat; (4) Liebermann-Burchardt for cholesterol; (5) Windaus for cholesterol; (6) polarized light for birefringent crystals of esterified cholesterol; (7) Kóssa for calcium; (8) Van Gieson for connective tissue; (9) del Rio Hortega for reticulin, collagen, and macrophages; (10) Lillie and Sylvén for metachromasia; (11) McManus (previous digestion with ptyalin) for glucoproteins, and (12) Hale (before and after hyaluronidase digestion) for mucopolysaccharides.[7,8] Grading on an arbitrary scale (0-4) was carried out in order to achieve a semiquantitative estimate of individual histologic and histochemical components of intimal aortic lesions (Tables 1 and 2).

Similarly, for detailed microscopic study of the coronary arteries, the myocardium of each specimen was sectioned 1 mm. below and parallel to the atrioventricular groove; two 8μ sections were then cut and stained by each of the aforementioned techniques. Examination of the stained sections was accomplished solely with light microscopy.

Results

Aorta Findings.—The normal chick aortic intima consists of a flat endothelium and a thin subendothelium, made up of a few reticulum fibers surrounded by a glucoprotein-staining material, which appears homogeneous at maximum magnification with the light microscope. As already indicated, aortic sections were made of the severest gross lesion in each segment, or of the central part of the segment when no gross lesion was visible. In the latter case, microscopic examination frequently revealed lesions. The gradings of individual histologic and histochemical components of these microscopic lesions are summarized for the four groups of chicks in Table 1. Comparison of control and experimental groups—i.e., Group 1 *vs.* Group 2 and Group 3 *vs.* Group 4—reveals few consistent significant differences. The data on foam cells, reticulum, collagen, cholesterol and its esters, calcium, metachromasia,

TABLE 3.—*Histochemical Findings in Sections of Grossly Normal Aortae*

Group	Total No. of Sections	No. of Microscopically Normal Sections	No. of Sections with Cholesterol-Lipid Deposition Only	No. of Sections with Cholesterol-Lipid Deposition Plus Other Histochemical Changes	No. of Sections with No Cholesterol-Lipid Deposition — Other Histochemical Changes Only
1					
2 C-O *	6	1	0	5	0
2					
2 C-O+estrogen prophylactic	4	0	1	3	0
3					
2 C-O	5	0	3	2	0
4					
2 C-O+estrogen therapeutic	6	1	2	3	0

* 2 C-O is 2% cholerterol plus 5% cottonseed oil.

glucoprotein, and mucopolysaccharides do not appear to reveal any definitive consistent differences. Basophilic cells and macrophages appear to be present in greater numbers in the estrogen-treated birds.

Analysis of the gradings for severe gross lesions reveals similar findings (Table 2). In accordance with the original reports,[1,2] gross aortic lesions were similar in distribution and degree in the paired groups. In these gross lesions, as compared with those detected by microscopy only, the number of basophilic cells, foam cells, and macrophages is increased. Reticulum and collagen fibers are thicker. The interstitial substances, cholesterol, glucoproteins, mucopolysaccharides, and metachromasia, are also increased. In agreement with the findings in the microscopically detected lesions, basophilic cells and macrophages were more abundant in the estrogen-treated birds (Groups 2 and 4).

A further analysis was made of the histologic and histochemical findings in the microscopic sections through grossly normal aortic segments (Table 3). In three groups of chicks, sections were identified which exhibited cholesterol-lipid deposition only, without other histologic-histochemical intimal alteration. In all four groups, lesions were noted which exhibited cholesterol-lipid deposition plus other histologic-histochemical changes. The combination —absence of cholesterol-lipid deposition and presence of other histologic-histochemical alterations—was *never* observed in any section from any group.

Coronary Findings.—The normal coronary artery of the chick has a flat endothelium lying immediately on the first medial elastic fibers.[3] There is no sharply defined internal elastic lamella. In some of the larger arteries a few thin reticulin fibers, surrounded by glucoproteins, may be found forming a subendothelium. The media shows poorly defined muscle cells, few elastic fibers, and some reticulin fibers, surrounded by glucoproteins. Between these cells, hyaluronidase-resistant mucopolysaccharides are present in varying quantity. There is a rather constant external elastic lamella. The adventitia gradually merges into the perivascular connective tissue and shows sparse elastic fibers, deriving from the external elastic lamella.

Fully developed atherosclerotic plaques in chick coronary arteries exhibit marked intimal thickening, resulting from large, clear, vacuolated cells filled with sudanophilic, cholesterol-positive material (Groups 1 and 3).[1-3] Between these cells reticulin fibers are found, surrounded by glucoproteins, and occasionally mucopolysaccharides. These plaques show a continuous endothelium and impinge on the lumen of the artery. The elastic fibers of the media are intact; sudanophilic material and choles-

terol may sometimes be found between these fibers.

As previously described, both groups receiving estrogens exhibited markedly different findings in the coronary arteries, as compared with their controls.[1,2] Atherosclerotic lesions were rare in estrogen-treated birds (Groups 2 and 4), whereas they were frequent in the controls (Groups 1 and 3). Estrogens had apparently reversed previously formed lesions, despite continued feeding of the potentially atherogenic diet (Group 4).[2] In the few lesions present in Groups 2 and 4, the special histologic and histochemical studies failed to reveal any differences, as compared with plaques in the control birds. In the predominantly normal coronary arteries of estrogen-treated Groups 2 and 4, histologic and histochemical studies revealed no alterations—i.e., in fibrils, cells, or ground substance—that might account for the virtual absence of atherosclerotic lesions. No morphologic basis in the coronary arteries was apparent for the phenomenon of estrogen-induced antiatherogenesis.

The histologic and histochemical studies revealed that most of the coronary arteries in the Group 4 birds were indistinguishable from normal vessels. They were generally devoid of detectable deviations from normal, e.g., in lipids, fibrils, cells, and ground substance.

Comment

Three aspects of these findings would seem to merit discussion. First, it was noted that macrophages and basophils were more abundant in the aortic lesions of estrogen-treated than in those of control birds. This finding may be related to the reported ability of estrogens to stimulate proliferation of the reticuloendothelial system.[9] It may be suggested, as a hypothesis or speculation, that increased basophilic and macrophage activity may be a factor in estrogen antiatherogenesis in the coronary arteries. The present data do not permit any real evaluation of this concept. It should be noted, however, that lesions were abundant in the aortas of estrogen-treated birds, despite increased macrophages and basophils. Contrariwise, these cells were not conspicuous in the coronary vessels, rendered virtually lesion free by estrogens; nor were they prominent in the few remaining lesions in the coronary arteries of Groups 2 and 4. Thus, this hypothesis or speculation seems at present very tenuous. Some preliminary data of the Chicago group[10] failed to reveal any augmented ability of the reticuloendothelial system to dispose of large particulate matter. No definitive suggestion emerged from the histologic-histochemical studies concerning the mechanism of estrogen action, so marked on the coronary arteries, and so lacking on the aorta.

Another significant finding was the invariable absence of other histologic-histochemical abnormalities—e.g., in ground substance—when cholesterol-lipid deposition was absent. This observation forcefully supports the concept that under conditions like those prevailing in these experiments—e.g., in young animals ingesting sizable amounts of cholesterol and fat—lipid-cholesterol deposition in the vascular intima is the first morphologic stage of atherogenesis. This deposition may, under suitable nutritional-metabolic circumstances, take place in otherwise normal, youthful vessels. No prior morphologic abnormality is a prerequisite for such deposition. This conclusion is, of course, in no sense inconsistent with the possibility that previous abnormalities of various types may facilitate lipid-cholesterol deposition in atherogenesis.

Finally, the essential normalcy of most of the coronary arteries in the Group 4 birds merits comment. This histologic-histochemical finding affirms that estrogens are capable of completely reversing the fibrillar, lipid, and other components of relatively "young" plaques. Atherosclerosis is, indeed, a totally reversible lesion—at least within limits.

Summary

In order to determine the temporal relationship between lipid-cholesterol deposition and other histochemical processes in the development and regression of atherosclerosis, sections of aortas and coronary arteries of chicks—sent as unknowns by the Chicago group—were examined with several staining methods by the Buenos Aires group.

The segmental difference between the estrogen effect on the aortic and on the coronary artery lesions of the chick was confirmed, but no insight was gained as to the cause of this difference. Estrogens are capable of completely reversing the fibrillar, lipid, and other components of relatively "young" coronary plaques in the chick, but have no such apparent effect on the aortic lesions.

The aortic lesions of estrogen-treated birds showed a greater abundance of macrophages and basophils than those of birds not so treated; this difference was not apparent in the coronary arteries.

The deposition of lipid-cholesterol can take place *de novo* in otherwise normal, youthful vessels when young birds are fed sizable amounts of cholesterol and fat. In this study, no prior morphologic abnormality was found to be a necessary prerequisite for lipid-cholesterol deposition.

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The Shwartzman Phenomenon in the Colon of Rabbits

A Serial Histological Study

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With the Technical Assistance of Jean Ablaza, B.A., and Harold Ford, B.A.

Introduction

The first observations on the phenomenon of local tissue reactivity apparently were made in 1894 by Sanarelli [1] while studying experimentally induced typhoid in monkeys. In a later publication,[2] the same author described a violent reaction in rabbits given a small dose of B. coli filtrate intravenously 24 hours after a well-tolerated intravenous injection of vibriones of cholera. Autopsy demonstrated a severe acute enteritis and nephritis. When the first injection was made in the sacculus rotundus or in the appendix, a severe general reaction ensued, and an acute local hemorrhagic inflammation was observed. Shwartzman made a thorough study of the local skin reactivity with bacterial filtrates [3-5] and established the conditions of the preparatory injection, the time interval, the reacting factors, and the relation of this phenomenon to other forms of tissue hypersensitivity. At the same time, Hanger [6,7] studied the sensitivity of rabbits to filtrates of Gram-negative bacteria predominating in their upper respiratory tract. He observed an intensification of the local skin reaction, with a local bluish discoloration and petechial hemorrhages, following an intravenous injection of the filtrate. Further contributions to the understanding of the Shwartzman phenomenon were made by Burnet,[8] Gratia and Linz,[9] and Bordet.[10]

The Shwartzman phenomenon has been studied in different organs of the experimental animal, including the knee joints,[11,12] lymph nodes,[13] adrenals,[14] eyes,[15] lungs,[5] kidneys,[5] stomach,[12,16] appendix,[17] and pancreas.[18] So far as we can determine, studies of the Shwartzman phenomenon in the large bowel have not been made. Therefore following previous studies with the Auer [19] procedure and the Arthus phenomenon,[20] the evolution of a local Shwartzman reaction in the colon was investigated in rabbits.

Materials and Methods

Thirty-four white New Zealand rabbits on a pellet diet and water ad lib. were operated upon to expose the bowel. Two-tenths cubic centimeter of a 1:10 dilution of a Serratia marcescens lysate was injected subserosally in the proximal and in the distal colon. Twenty-four hours later, 1 cc. of a 1:2 dilution of the same lysate was injected intravenously. Animals were killed at the following times subsequent to the eliciting injection: 2, 3, 6, 7, 24, 32, 48, 72, 96, 120, 144, 192, 216, 240, 264, 288, 504, and 672 hours. The original number of rabbits was larger, but some were discarded because they were found dead and autolysis prevented adequate study of the colon.

Several control procedures were utilized. One group of animals received injections of isotonic saline into the proximal and distal colon, and 24 hours later a 1:2 solution of S. marcescens lysate was given intravenously. In a second series, one site was prepared with saline, the other with lysate; the eliciting injection of 1:2 lysate was given 24 hours later. A third group of animals received preoperatively 2 cc. of a 1% solution of formalin as a mild irritant rectally. At operation 0.2 cc. of lysate or 0.2 cc. of saline locally was injected, the eliciting injection being omitted. In

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Gross and Microscopic Findings of Shwartzman Reaction in Large Bowel

Animal No.	Sex	Hour	Gross Reaction	Gross Findings	Microscopic Findings
101	M	2	Slight positive	Blood clot on 1 of 2 sites	Edema, engorgement of capillaries, hemorrhagic and leukocytic infiltration (Figs. 1, 2); hemorrhagic infarction of entire wall (Fig. 3); degeneration of epithelial lining of Lieberkuhn's glands (Fig. 4); heavy leukocytic infiltration with abscess-like collections (Fig. 5)
72	M	3	Slight positive	Hyperemia of proximal colon	Hyperemia and cellular infiltration in the serosa and lamina propria; predominance of leukocytes
74	M	6	Slight positive	Mild hyperemia at both sites	Infiltration of the submucosa with polymorphonuclears and erythrocytes; increased production of mucus; in other sites, edema only
37	M	6	Negative	--	Severe cellular infiltration of the submucosa; hyaline deposits in vascular wall (Fig. 6) infiltrated by leukocytes; degeneration of muscularis with focal accumulations of leukocytes
34	M	7	Positive	Hemorrhage at 1 of 3 sites	Cellular-vascular proliferation in the submucosa (Fig. 7); severe hemorrhage in submucosa, with destruction and ulceration of mucosa; infiltration of serosa and muscularis propria, with necrosis; hemorrhage in the serosa thickened by histiocytic proliferation
35	F	7	Strongly positive	Marked hemorrhages with blisters in 2 of 3 sites	Severe hemorrhage with destruction of mucosa; cellular reaction in serosa with adhesion to adjoining bowel; muscularis infiltrated with eosinophils
36	F	7	Strongly positive	Hemorrhages with blisters in 2 or 3 sites	Localized hemorrhagic and leukocytic infiltration of entire thickness of the bowel wall; marked reaction of the mesocolon with 2 thrombosed vessels; thrombosis and perivascular proliferation (Fig. 8)
38	M	7	Strongly positive	Hemorrhages and blisters in 2 of 4 sites (Fig. 9)	Foci of hemorrhage in the muscularis and submucosa with destruction of mucosa (Fig. 10); histiocytic reaction around the hemorrhage; outside these foci, bowel unaffected.
73	M	12	Strongly positive	Both sites with hemorrhages	Submucosa widened with cellular infiltrates; histiocytes and leukocytes mainly, the latter collected in small abscesses; dilated lymphatics, no hemorrhage
31	M	14	Strongly positive	Marked injection and hemorrhage over a 4 inch area	In both the proximal and distal colon: Hemorrhage in submucosa; edema, lymphatic dilatation, and cellular infiltration in the submucosa; occasional vascular thromboses
75	M	24	Strongly positive	Hyperemia at both sites, with blister proximally	Spectacular edema, dissociating all structures; diffuse cellular infiltration, predominantly leukocytic
100	F	24	Strongly positive	Pronounced hyperemia extending 2-3 cm. from injection site	
176	M	24	One slightly, the other strongly, positive	Hyperemia and hemorrhage	Extensive infiltration of mononuclears and leukocytes; vascular thrombi
182	M	24	Strongly positive	Hemorrhage at both sites	Proximal colon: Hemorrhage in the submucosa; ulceration of mucosa; cellular infiltration with destruction of muscularis Distal colon: Marked edema, with lymphatic dilatation in the submucosa; local accumulation of polymorphonuclears; hemorrhages in mucosa and submucosa
97	M	32	Strongly positive	Hemorrhage at proximal site	Proximal site: Severe reaction with vascular thrombosis; hemorrhagic infiltration with destruction of a wide area of muscularis (Fig. 12); marked reaction in serosa with leukocytes and mononuclears Distal site: Multiple thromboses in venules and capillaries; arteries intact; degeneration of muscularis propria with leukocytic infiltration; thickened and cellular serosa and submucosa (Fig. 13)
76	F	48	Strongly positive	Both sites hemorrhagic	Venous thrombosis, muscle degeneration, and infiltration of submucosa; no pronounced changes in proximal site
77	F	72	Strongly positive	Hemorrhage in one site and a nodule in the other	Destruction of muscle by exudate; vascular thromboses; cellular infiltration; fibroblastic proliferation At the other site: Extensive hemorrhage and cellular infiltration in the muscularis, serosa, and submucosa, with secondary destruction and ulceration of the mucosa
81	F	72	Strongly positive	Ulceration and nodule in proximal colon	Vascular thrombosis; degeneration of muscle with deep purple staining; acute inflammatory exudate and fibrocellular proliferation interrupting continuity of this layer (Fig. 14)

Gross and Microscopic Findings of Shwartzman Reaction in Large Bowel—Continued

Animal No.	Sex	Hour	Gross Reaction	Gross Findings	Microscopic Findings
79	F	96	Strongly positive	Nodule in proximal colon; hyperemia at both sites	Submucosa infiltrated mostly with mononuclears; thickened and inflamed serosa with foci of necrosis of the cellular infiltrate; fibroblastic proliferation and formation of new capillaries; thrombosis of vessels; proliferation of fatty tissue trabeculae; similar finding at the other site
80	F	120	Positive	Nodules at both sites	Extensive infiltration and necrosis of muscle layer with deposits of dark-purple-stained nuclear debris (Fig. 15); less extensive, but similar, phenomena in the submucosa; mucosa only slightly infiltrated, edematous At the other site: Thickening and mononuclear infiltration
120	F	120	Slightly positive	Nodule in proximal colon	Extensive lesion in the submucosa with diffuse histiocytosis of submucosa; remnants of hematoma replaced by histiocytic and monocytic cells; in distal colon fibrosis of serosa only
121	F	120	Positive	Nodule and hyperemia at proximal colon	Fibrosis of submucosa at proximal site, which is thick and infiltrated by monocytes and fibroblasts
122	F	144	Slightly positive	Nodule in proximal colon	Fibrosis of thickened serosa; fibrosis of interstitial tissue of muscularis propria; same in distant colon with less fibrosis
98	M	192	Positive	At both sites "pustular"-like formation	Thickening of mesocolon and of colonic serosa, with fibrosis and formation of a cellular-vascular tissue; leukocytes, sparse Distal colon: Foci of muscle degeneration; necrotic masses of purple-stained material with surrounding granulomas; localized thickening and fibrosis of the serosa, with giant cells
125	F	216	Slightly positive	Nodule at proximal site	Granulomas in the serosa around double refractile crystals At proximal site: Foci of hemorrhage in submucosa and subserosa with destruction of muscularis; fibrotic capsule around lesion; focal accumulations of lymphocytes and monocytes; bowel wall four times its normal thickness at this level
124	F	240	Slightly positive	Two nodules in the proximal colon	At proximal site: Granulomas with giant cells; subserosal, large hematoma with thick fibrotic capsule At distal site: Focal infiltration of lymphocytes and monocytes adjoining a large granulomatous proliferation, with giant cells and tissue eosinophils (Fig. 16) Focal infiltrations of lymphocytes at base of the crypts
117	F	264	Negative	--	Proximally: Granulomata and giant cells in subserosa (Fig. 17) Distally: Large hematoma in the submucosa Edema of mucosa with hemorrhagic infiltration
118	F	288	Slightly positive	Nodule in proximal colon	At proximal site: Foci of hemorrhage with a surrounding cellular reaction; cellular infiltration and fibrosis of submucosa At distal site: Subserosal focus of granulomatous inflammation with necrotic debris and giant cells
181	F	3 wk.	Negative	--	Proximally: Collagenosis of external layer of muscularis propria Distally: Focal collections of lymphocytes Diffuse histiocytosis; widened submucosa with perivascular infiltrates
178	F	3 wk.	Negative	--	Proximally: Negative Distally: Collections of lymphocytes and histiocytes in the serosa; cellular infiltrates in the muscularis; perivascular infiltration in submucosa
184	F	3 wk.	Negative	--	Proximally: Nodule of granulomatous tissue in serosa; collections of lymphocytes and plasma cells; no giant cells seen. Distally: Normal colon
180	F	4 wk.	Negative	--	Proximally: Negative. Distally: Perivascular proliferation in the serosa; muscularis with darkly stained degenerative area and granuloma formation
183	M	4 wk.	Negative	--	Proximally: Focal infiltrations of lymphocytes in the lamina propria; area of fibrosis in the serosa with granuloma and giant cell formation Distally: Negative
174	F	4 wk.	Negative	--	Negative
175	F	4 wk.	Negative	--	Proximally: Negative Distally: Granulomatous inflammation in the serosa; muscularis proliferating into the serosa

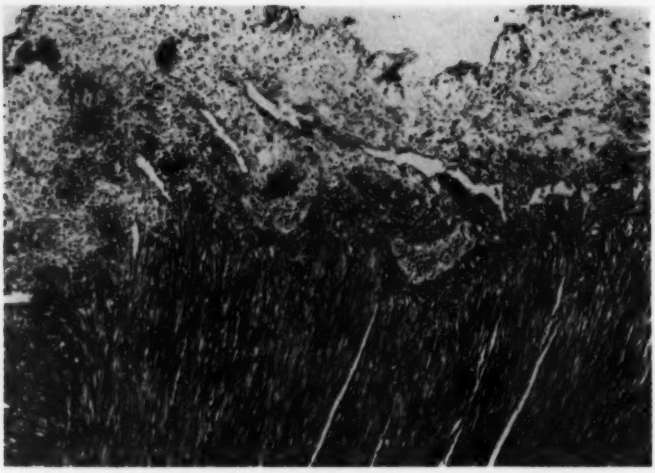

Fig. 1.—Rabbit No. 101, male, Serial Shwartzman (two hours). Edema of the serosa; engorgement of capillaries; hemorrhagic and diffuse leukocytic infiltration. Reduced to ⅔ of mag. × 96.

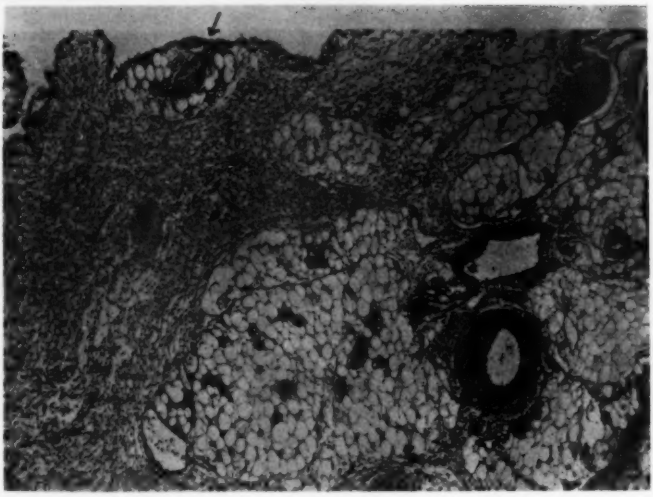

Fig. 2.—Rabbit No. 101, male, Serial Shwartzman (two hours). Cellular infiltration of mesocolon, mostly leukocytes. Larger vessels appear normal. Smaller vessels are engorged with erythrocytes and leukocytes, the last in marginal arrangement (arrow). Reduced to ⅔ of mag. × 69.

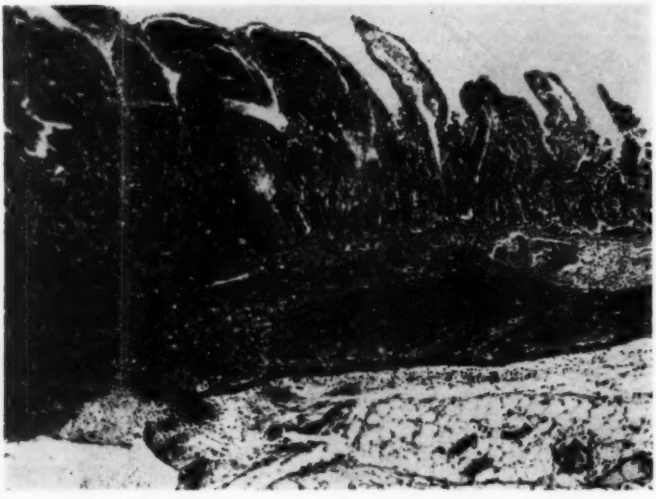

Fig. 3.—Rabbit No. 101, male, Serial Shwartzman (two hours). Hemorrhagic infiltration of entire intestinal wall over a large area. Reduced to ⅔ of mag. × 75.

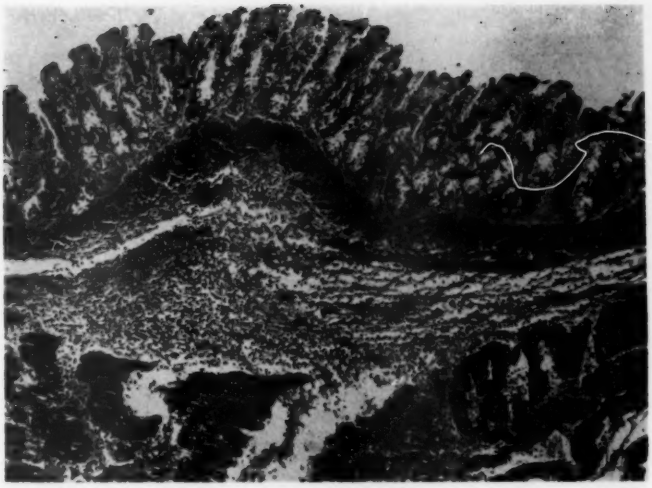

Fig. 4.—Rabbit No. 101, male, Serial Shwartzman (two hours). Degeneration of epithelial cells, edema, and cellular infiltration of submucosa with leukocytes and mononuclear cells. Reduced to ⅔ of mag. × 89.

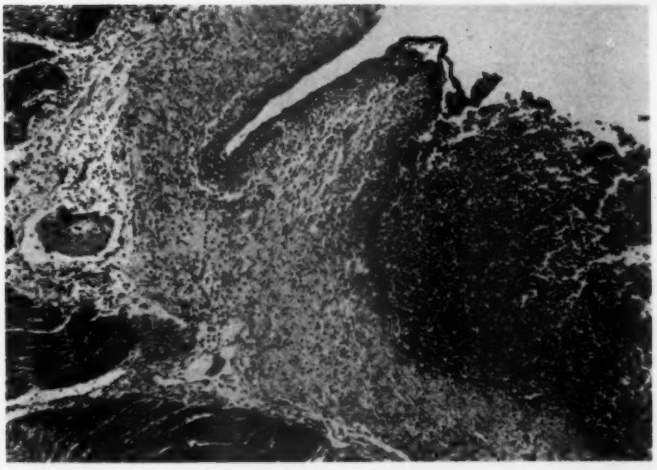

Fig. 5.—Rabbit No. 101, male, Serial Shwartzman (two hours). The cellular reaction in the serosa may reach proportions of a localized abscess. Reduced to ⅔ of mag. × 100.

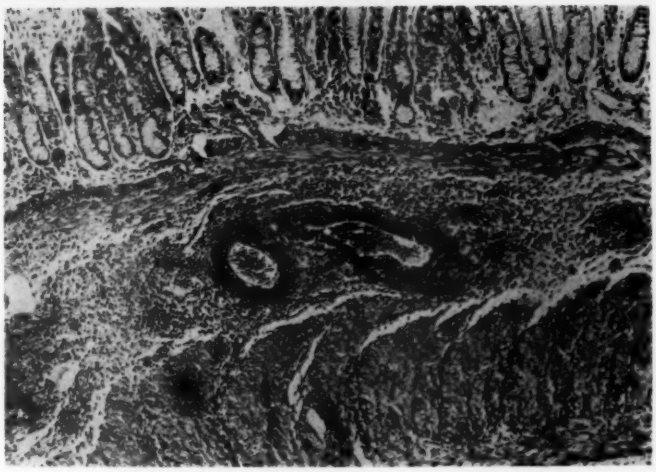

Fig. 6.—Rabbit No. 37, male, Serial Shwartzman (six hours). Vascular walls contain hyalin deposits. Submucosal infiltration mainly by polymorphonuclear cells. Reduced to ⅔ of mag. × 110.

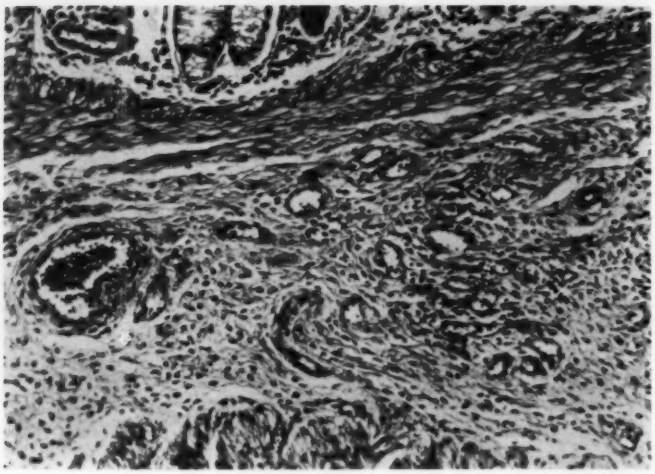

Fig. 7.—Rabbit No. 34, male, Serial Shwartzman (seven hours). Cellular proliferation in the submucosa. This reaction seems older than the chronological age of the lesion would suggest. Reduced to ⅔ of mag. × 210.

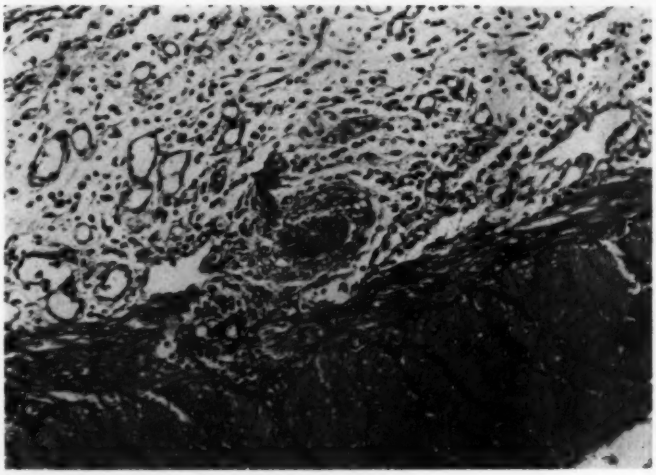

Fig. 8.—Rabbit No. 36, female, Serial Shwartzman (seven hours). Thrombosed vessel. Perivascular proliferation and formation of new capillaries. Reduced to ⅔ of mag. × 220.

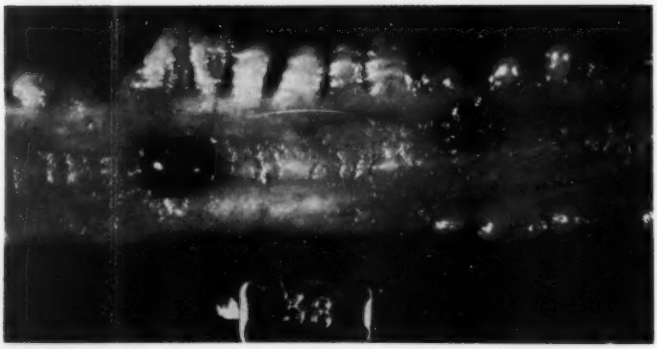

Fig. 9.—Rabbit No. 38, male, Serial Shwartzman (seven hours). Strongly positive reaction. Hemorrhagic areas with blister formation.

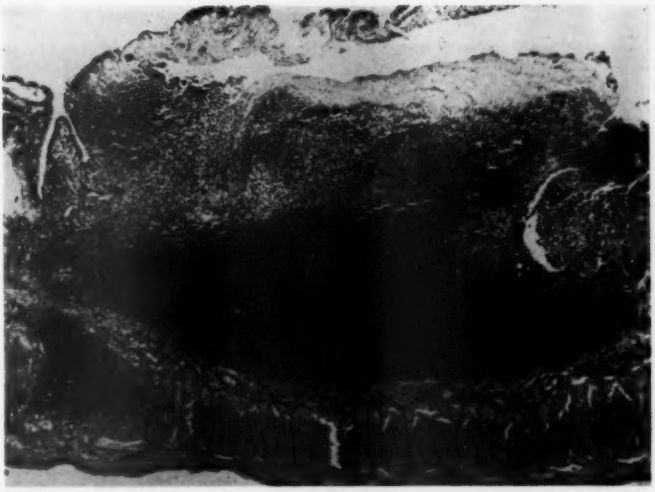

Fig. 10.—Rabbit No. 38, male, Serial Shwartzman (seven hours). Foci of hemorrhage in the muscularis and submucosa with destruction of the mucosa. Reduced to ⅔ of mag. × 64.

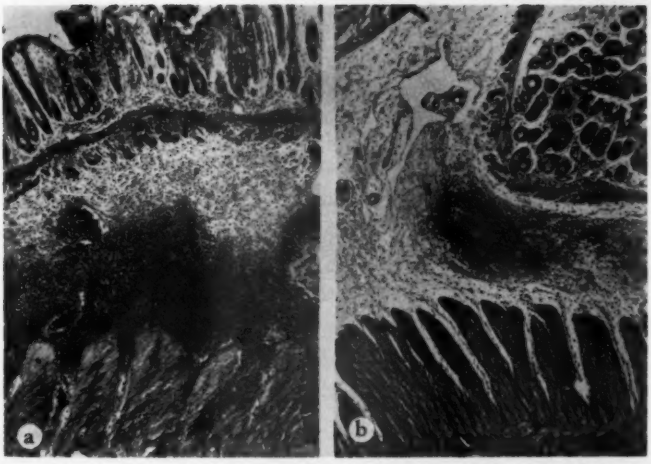

Fig. 11.—Rabbit No. 129, female (control), Serial Shwartzman (24 hours). (*a*) Site of S. Marcescens injection; note 2 thrombosed vessels. (*b*) Site of saline injection. Reduced to ⅔ of mag. × 55.

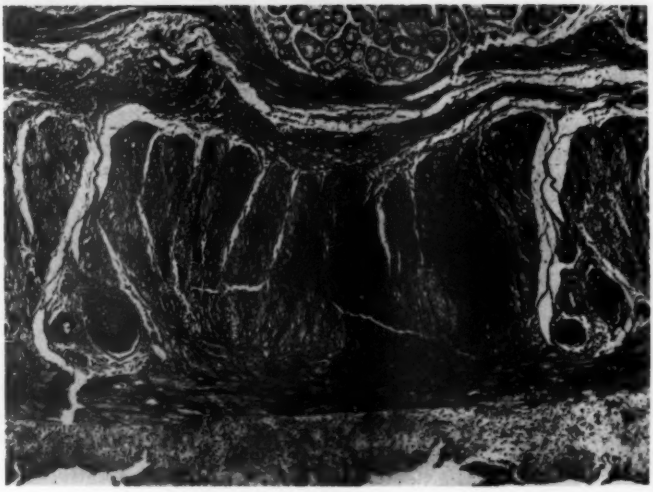

Fig. 12.—Rabbit No. 97, male, Serial Shwartzman (32 hours). Multiple thromboses in venules; degeneration of muscularis with polymorphonuclear and hemorrhagic infiltration; thickened and infiltrated serosa and submucosa. Reduced to ⅔ of mag. × 74.

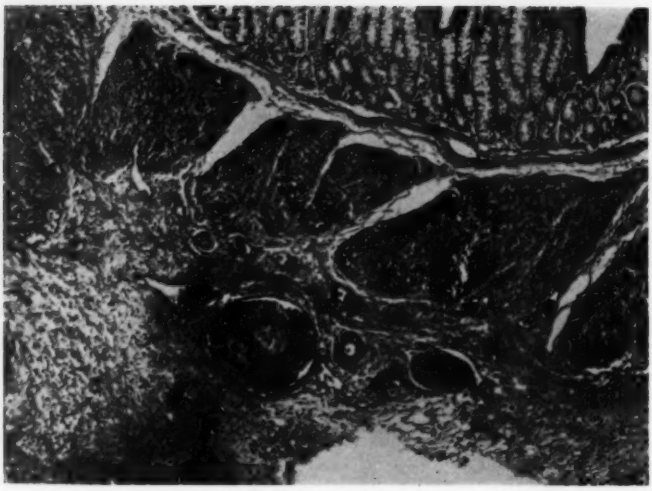

Fig. 13.—Rabbit No. 97, male, Serial Shwartzman (32 hours). Vascular thromboses, muscular degeneration. Infiltration of serosa with polymorphonuclears and monocytes. Reduced to ⅔ of mag. × 90.

two animals multiple sites were injected to test the reactivity of different areas of the bowel, and also to approach conditions as they might occur in natural disease, as suggested in a previous paper.[21] The experiments were concluded at the appropriate time by a lethal dose of barbiturate; the abdomen was reopened; the sites of injection were inspected and described. Tissues were fixed in buffered formalin; blocks in paraffin were cut, and the sections were stained, chiefly with hematoxylin and eosin.

Results

The gross and microscopic findings are presented in the Table. No positive findings were observed in the controls, except for the frequent presence of a small hemorrhage at the injection site. Microscopically, in the control cases there was edema and a traumatic hemorrhage, with some edema, but no evidence of inflammation. Figure 11 illustrates the contrast between sites prepared with lysate and saline.

Comment

The histopathology of the Shwartzman phenomenon has been the subject of extensive study.[22-28] These observations usually

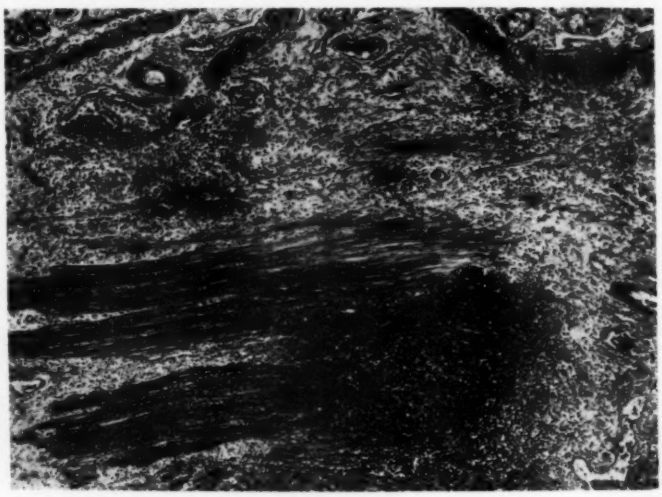

Fig. 14.—Rabbit No. 81, female, Serial Shwartzman—single site (72 hours). Vascular thromboses: destruction of muscularis by infiltration of polymorphonuclear cells. Note replacement by young granulation tissue. Reduced to ⅔ of mag. × 88.

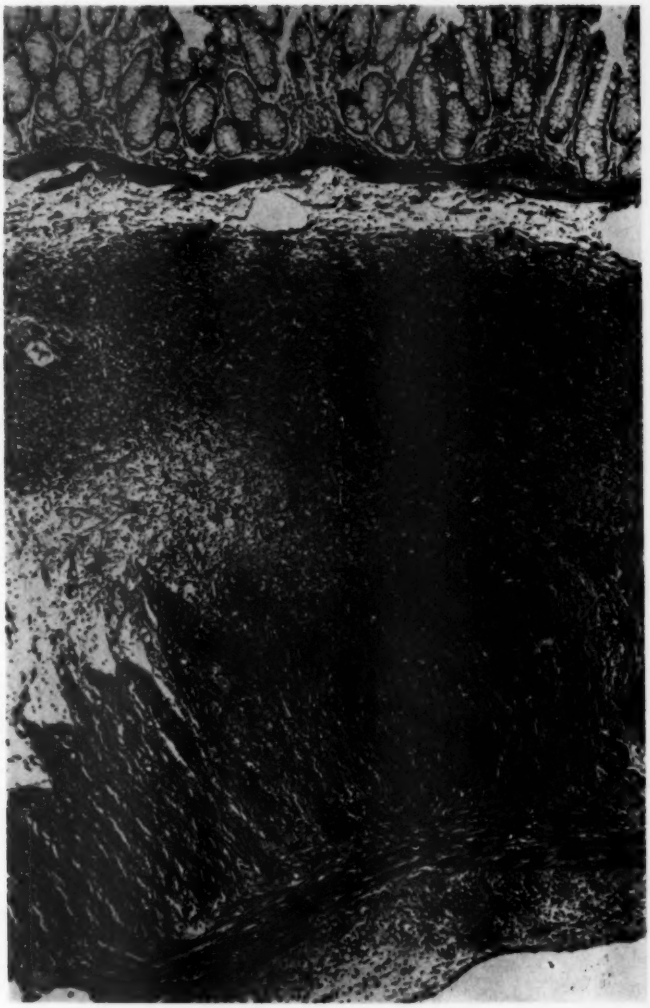

Fig. 15.—Rabbit No. 80, male, Serial Shwartzman (120 hours). Necrotic and hemorrhagic lesion in the submucosa and muscularis.

were concerned with the early phases of the reaction, and only a few [12,23,26] included tissues more than 24 hours after the challenging injection. A controversy exists as to the findings after the preparatory phase,[25,26] but since this problem was not investigated in the present experiments, only the findings of the Shwartzman phenomenon after the eliciting injection will be considered.

The reaction has been pronounced and severe in the two- and three-hour preparations, a feature emphasized by other observers. For clarity, the various aspects of the reaction are discussed individually.

Edema.—This feature, present at the first two-hour observation, was again prominent in six-hour, and especially in 24-hour, preparations. Later, it appeared to be a secondary accompaniment of the existing inflammation and was observed only rarely, as in the 120- and 163-hour specimens. Edema does not seem to be a primary fea-

ture of the Shwartzman phenomenon, in contrast to the Arthus reaction.[20]

Cellular Infiltration.—Predominantly polymorphonuclear in the first seven hours after the eliciting injection, these cells seem to yield gradually to a mononuclear type of cellularity. In some sections, mononuclear cells may be prevalent in 12-hour specimens, while they were in equal proportions with leukocytes in the 32-hour specimens. In the three-day specimens, fibroblasts seem numerous, and they become more prevalent two or three days later. Lymphocytes and plasma cells are more numerous at the same time and are constantly seen at the bases of the crypts.

Vascular Damage.—Hyaline deposits in the vascular wall were observed in the seven-hour specimens and occasionally during the entire course of the serial observations. Arteries of small and medium size, nevertheless, were conspicuous by maintaining their integrity in the midst of pronounced reactions. More attention was directed to capillaries and veins. The former frequently were widely dilated and

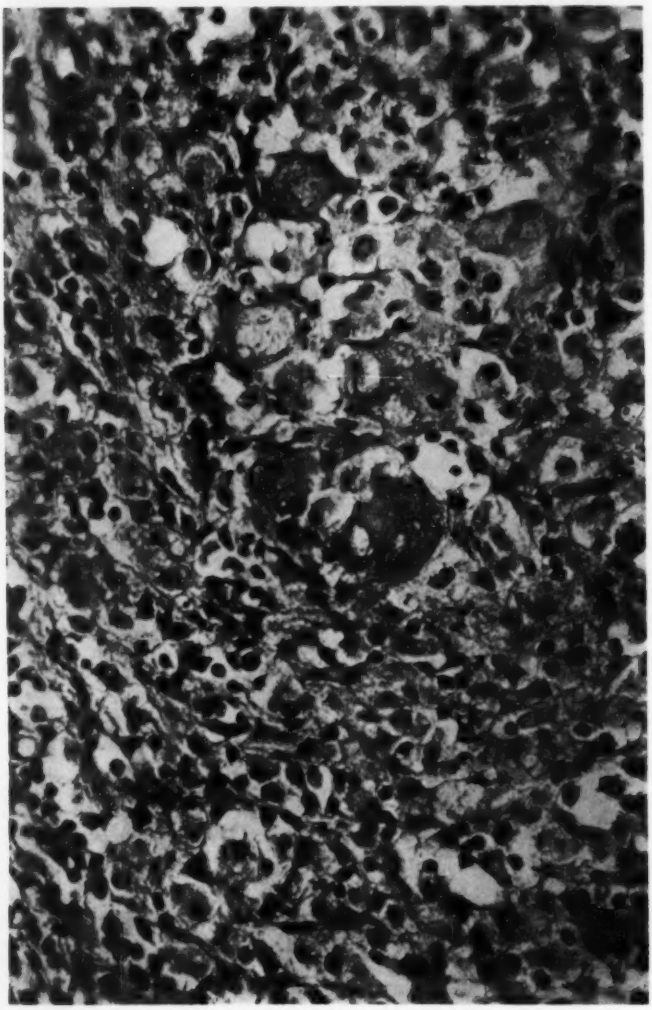

Fig. 16.—Rabbit No. 124, female, Serial Shwartzman (240 hours). Granulomata and giant cells; diffuse tissue eosinophilia. × 500.

engorged with erythrocytes; the latter contained surrounding foci of inflammatory cells, mainly leukocytes.

Venous and Capillary Thrombosis.—By far the most important feature of the Shwartzman phenomenon was vascular thrombi. Seen as early as on the 7-hour specimens, they were spectacular in the 32-, 48-, and 72-hour preparations. Thrombi appeared as purple masses filling the vascular lumen, with an admixture of a few leukocytes. The vascular endothelium appeared quite normal. Since the peak of the reaction was at approximately the 24th hour and the peak of thrombotic reaction 1 or 2 days later, the primary role of thrombosis in causing tissue necrosis remains doubtful. Thrombi were rare 72 hours after the challenging injection and were not seen in specimens 1 to 4 weeks old.

Hemorrhage.—This is a very important feature of the Shwartzman phenomenon. The cause of the hemorrhage remains un-

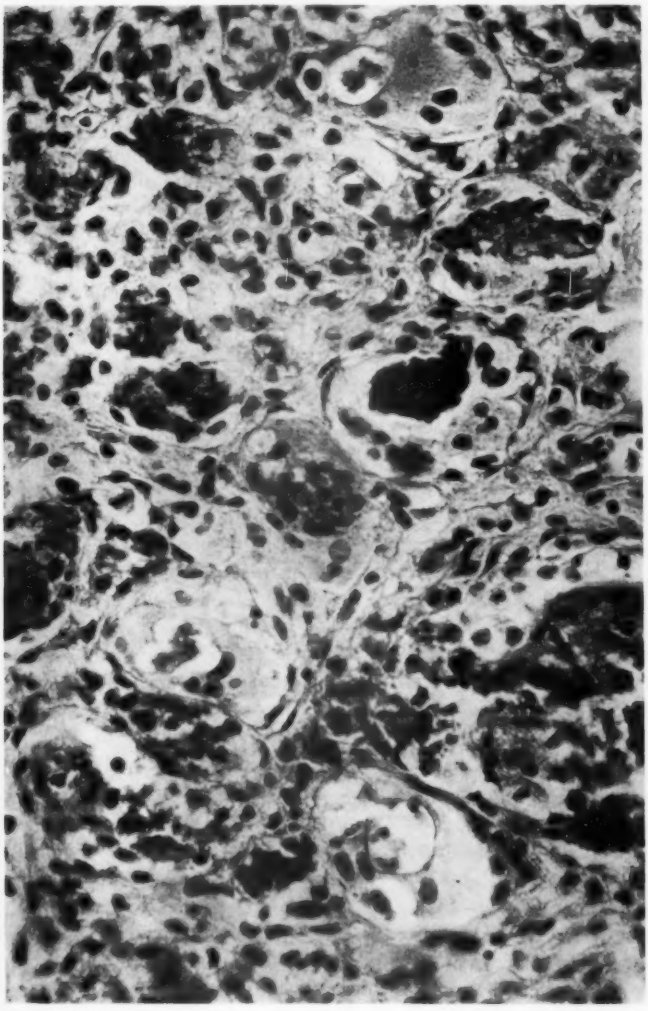

Fig. 17.—Rabbit No. 117, female, Serial Shwartzman (264 hours). Necrotic debris, granulomata and giant cells. × 500.

certain. Some observers regard damage to vascular endothelium as the cause of bleeding.[22,24] Others could not confirm such findings.[20,28] In the present material there was no obvious site of damage in arterioles or large arteries. Large foci of hemorrhage dissecting the overlying structures were seen, suggesting a rather localized lesion from a torn vessel. The evidence for such an event in the early phase of the Shwartzman phenomenon seems difficult to obtain. Hemorrhages are almost constant in this reaction and vary from large hematomata to diffusely infiltrating hemorrhagic infiltration. Hemorrhagic phenomena could be observed at the earliest stage of observation and later in 9-, 11-, and 12-day-old specimens. No hemorrhages were observed in the three- and four-week preparations.

Granuloma and Giant-Cell Formation.

This feature has not been described previously as a characteristic of the Shwartzman phenomenon. Granuloma formation was observed repeatedly in 9-, 10-, 11-, 12-, 21-, and 28-day specimens. Gerber [26] is the only author to have reached the four-week limit in his serial observations. The findings after the seventh day in his material were those of a nonspecific granulation tissue. Our interest was more for the later events in the local reaction, and this may account for the unusual rich yield of granulomas. The latter usually are considered an expression of a hypersensitive state [29] and their presence would favor the opinion of those who, contrary to Shwartzman,[5] consider the Shwartzman phenomenon an anaphylactic reaction.[2,6,9,15]

Muscle Necrosis.—This appeared as early as six hours after the challenging injection. In most cases these necroses were observed in conjunction with hemorrhage in the muscularis, but sometimes independently of hemorrhage and for an extension beyond the local effect of the preparing injection. Muscle necrosis appeared before vascular thrombosis, suggesting that it is a primary phenomenon of the Shwartzman reaction. The muscle fibers in such cases appeared interrupted in their continuity and stained purplish in the hematoxylin-eosin-stained sections. Collagenosis of the external muscularis, observed in the Arthus reaction,[20] has been noted occasionally in this material. Muscle fibers have been observed to fan out into the serosa in four-week specimens, denoting a proliferative stimulus operating in an otherwise unsuspected lesion.

Tissue Eosinophilia.—This feature has not been outstanding in the Shwartzman phenomenon. It was observed once in the 7-hour specimen as part of the early response, and in the 10-day preparation, together with granulomas.

Negative Gross Findings Versus Positive Microscopic Findings.—On many occasions gross findings were absent or minimal (Animals 37, 122, 117, 118, and all 3- to 4-week specimens), while the microscopic examination revealed pronounced changes. Similarly, in human disease, grossly negative findings by inspection or palpation should be corroborated by microscopic observation to rule out latent pathology.

Pathological Findings in the Control Group.—The findings in sites injected with S. marcescens, compared with those in other sites prepared with isotonic saline, were very instructive. The differences were striking because the thrombi, and inflammation present in the test sites, were not demonstrable in the control areas. In other cases the changes observed were coccidiosis of the colon, localized ulceration with a polyp of inflammatory tissue, and diffuse infiltration of the lamina propria in single cases, each. Fibrosis of the lamina propria occurred on three occasions in animals given formalin enemas. No thromboses, granulomas, or muscle degenerations were observed in the control animals.

Significance of the Shwartzman Phenomenon for Human Disease.—Many observers [9,10,15,17,22,24] have expressed the opinion that the Shwartzman reaction is not merely an experimental curiosity, but may occur in man and explain the pathogenesis of cer-

tain obscure diseases. This topic is considered in a separate paper.[30]

Comparison of the Arthus Reaction and the Shwartzman Phenomenon.—This has been made frequently, with opinions varying from a local hypersensitivity reaction to complete independence of the two phenomena. In a more recent study, Stetson [31] found many features of the Shwartzman phenomenon in advanced stages of the Arthus reaction, an idea expressed almost 20 years earlier by Apitz.[23] Both reactions point, as do Koch's phenomenon and general anaphylaxis, to a tendency of the organism to react in different ways to the introduction of foreign substances.

Summary and Conclusion

The local Shwartzman phenomenon was studied in the colon of rabbits in which a solution of Serratia marcescens lysate had been given into the intestine through a transperitoneal approach and the same lysate had then been injected 24 hours later intravenously. The colon responded with a violent local reaction, characterized grossly by hyperemia and hemorrhage, and microscopically by hemorrhage, thrombosis, and, later, granuloma formation.

The colon of rabbits reacts to the local Shwartzman procedure with hemorrhage, vascular thrombosis, necrosis, and acute and chronic inflammation; granulomata are demonstrable in the late stages of the reaction.

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Primary Dissecting Aneurysms of Peripheral and Pulmonary Arteries

Dissecting Hemorrhage of Media

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Dissecting aneurysms of the aorta are relatively common. In their complete review of the literature written in the English language, in the period from 1933 to 1953, inclusive, Hirst, Johns, and Kime[1] found an incidence of 0.28%, or 1 in every 363 cases in 31 reported series, representing a total of 175,405 reported autopsies. Extension of hemorrhage in the media of dissecting aneurysms of the aorta into major branches occurs frequently, often leading to symptoms of occlusion of the artery involved. However, primary dissecting aneurysms of pulmonary and peripheral arteries without involvement of the aorta are rare. By definition we mean a lesion in which there is a dissecting hemorrhage in the media, splitting the wall for a variable distance at some level between the internal and the external elastic lamina of the artery. We, of course, have not concerned ourselves in this paper with intimal hemorrhages in or about atheromatous plaques, such as one sees commonly in sclerotic coronary arteries, and which have been described so well by Paterson.[2]

Only 31 cases in which the gross or microscopic findings or both appear adequate for classification as dissecting aneurysms have been reported in the literature in the last 34 years. Nearly all of these represent single case reports, some in journals of clinical specialties; and in some instances features of pathologic interest are omitted. A short summary of the clinical and pathologic findings in these 31 cases is given in Table 1. We have left out several cases reported under the title of dissecting aneurysm because of incomplete detail of pathologic description. References to eight cases in the older literature previous to 1924 are given in Shennan's[3] monograph on dissecting aneurysms of the aorta, and some of these cases are included in Watson's[4] more recent article. In these cases the following arteries were involved: pulmonary, three cases; subclavian, two cases; thyroid, two cases; external iliac and femoral, each one case. Two other pulmonary artery cases, one reported by Helmbrecht, in 1842, and one by Giordano, in 1904, are cited by D'Aunoy and von Haam[5] in their comprehensive review of pulmonary artery aneurysms in 1934.

During the years 1931-1957, inclusive, 11 Huntington Memorial Hospital patients with primary dissecting aneurysm of arteries other than the aorta were autopsied. In all of these the aorta was free from intramural hemorrhage or dissection. During the same period there were 32 cases of dissecting aneurysm primary in the aorta. Nearly all of these showed extension of hemorrhage into one or more major branches of the aorta. In this period there was a total of 6,666 autopsies, including 1,243 stillborn and neonatal deaths. Several local confreres have courteously furnished

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TABLE 1.—*Dissecting Aneurysms of Arteries Other*

Case	Author Year	Artery	Age	Sex	Blood Pressure	Days Duration	Extent of Dissection	Thrombosis	Infarction	Intimal Tear
1	Liebow, Cline, et al.[6] 1956	Left renal & branches; 2 of 3 rt. renal branches	57	M	140/100	7	4+	0	1+	0
2	Gilfillan, Smart, & Bostick,[7] 1956	Left renal and branches (surgical)	47	M	132/82	12	4+	+	+	0
3	Watson[4] Case 2 1956	Right renal	52	M	?	1	30 mm.	0	0	+
4	Case 3 1956	Main branches of both renals	49	F	190/90	12	3+ rt.; 2+ lt.	0 rt.; 0 lt.	2+ rt.; + lt.	+ rt.; + lt.
5	Boyd and Watson[8] Case 2 1956	Right renal branch	58	M	220/130	9	+	0	+	0
6	Glendy, Castleman, & White[9] 1937	Left anterior descending coronary	51	M	?	5	4 mm.	0	+	?
7	Uehlinger[10] Case 1 1947	Left ant. descend. coronary	46	M	?	1½ hr.	5 mm.	+	0	0
8	Case 2	Left ant. descend. coronary	45	M	?	8	+	0	+	+
9	Hedinger[11] 1947	Left ant. descend. coronary	53	M	?	9	5 mm.	+	+	+
10	Lovitt and Corzine[12] 1952	Left ant. descend. coronary	39	F	140/80	2 hr.	4+	0	0	0
11	Scholefield[13] 1924	Right vertebral and basilar	47	M	150 ?	15	4+	?	?	?
12	Hyland[14] Case 2 1933	Basilar	42	M	?	19	+	+	+	?
13	Szabo[5] 1939	Right vertebral (intracranial)	35	F	?	40	18 mm.	0	0?	0
14	Dratz and Woodhall[15] 1947	Left internal carotid and cerebral branches	21	F	?	2½	3+	0	+	0
15	Sinclair[17] 1953	Right middle cerebral and branches	27	F	118/80	3	20 mm. Br. 15 mm. 20 mm.	0	+	0

Than Aorta: Cases Reported in Literature Since 1924

Clinical Summary	Anatomic Findings
Acute bilateral constant costovertebral pain; hematuria; progressive oliguria; increase of blood urea N to 216 mg. %; death from uremia with convulsion on 7th day	Extensive dissection of media of left renal and of 2 of 3 major branches of right renal artery, far into kidneys; marked narrowing of lumens; bilateral renal infarcts; moderate aortic arteriosclerosis
Sudden intense left flank pain; moderate shock; mild hematuria; pyelogram showed no function in left kidney; nephrectomy 12 days after onset; patient well 4 yr. later	Dissecting hemorrhage in media from level 2 cm. beyond aorta into branches in kidney; small thrombus in narrowed lumen; accessory artery to upper pole free; extensive renal infarction; surgical specimen
Headaches for 2 wk.; sudden onset of confusion and later coma; spinal fluid very bloody; pressure 300 mm. of water; death 24 hr. after onset of coma	Dissection of right renal artery for 3 cm.; branches free; no renal infarction; hypertrophied heart—450 gm. (hypertensive?); recent hemorrhage in midbrain extending to meninges; bloody fluid in cerebral ventricles
Previous Raynaud's phenomenon; sudden onset of severe pain in right, and later in left, loin; ankle and sacral edema; trace of albumin and granular casts in urine; blood urea 62 mg. %; death in uremic coma 12 days later	Major branches of both renal arteries showed dissecting hemorrhage, right more than left; main trunks free; extensive infarction of right kidney, focal necrosis in left; malignant nephrosclerosis; hypertensive heart, 400 gm.
Dyspnea on exertion for some months; B.P. 220/130 mm.; a few weeks later right hemiparesis; large retroperitoneal tumor found; right renal artery dissected from posterior surface; rapid decline; clinical evidence of myocardial infarct, coma, and death 9 days postoperative; blood urea 388 mg.	Dissecting aneurysm of upper major branch of right renal artery; recent infarct of upper pole of kidney; massive retroperitoneal ganglioneuroma; recent anterior infarct in hypertensive heart—470 gm.; recent thrombosis in anterior descending coronary artery; recent softening of temporal lobe of left cerebrum
Slowly progressing cardiac insufficiency for 10 yr.; cardiac enlargement; rapid congestive failure in last 5 days; clinically arteriosclerotic heart disease; patient died in 1902	Slit 4 mm. long, filled with clotted blood in wall of left descending coronary artery; right auricular and left ventricular thrombi; cardiac hypertrophy; subacute glomerulonephritis
Severe exercise (military duty) in extreme cold 5 days before death; sudden death 1½ hr. after onset of severe cardiaci llness	Fresh dissecting hemorrhage in media in segment 5 mm. long in midportion of left descending coronary artery, nearly closing lumen; recent thrombus in 2 cm. long segment distal to aneurysm; heart dilated; too early for changes of infarction
Strenuous exercise (military duty) in last weeks of life; severe fatigue 8 to 10 days before death; clinical course that of coronary occlusion plus cerebral symptoms	Spindle-shaped segment in midpart of left descending coronary artery showed dissection and complete occlusion; large anterior cardiac infarct; mural thrombus in left ventricle; left middle cerebral embolism with cerebral softening
Kicked by horse, fracturing sternum and 2 costal cartilages; developed hemorrhage over sternum, marked dyspnea, and pericardial friction rub; sudden death	Tear of intima and media with dissection of wall for short distance just below origin of artery; contusion and anterior myocardial infarct present; hemopericardium (500 cc.) due to rupture of infarct
Sudden onset of severe spontaneous chest pain accompanied by mild dyspnea and cyanosis 14 days after normal delivery of full-term child; severer pain 2 hr. later; death 2½ hr. after onset	Dissection of media in entire length of left descending coronary artery extending into apical branches; lumen occluded; death too early for observable changes of infarction
Gradual onset of cerebral symptoms, including severe headache, inability to swallow, and paralysis of right vocal cord and right palate; difficulty of breathing developed; terminal pneumonia ensued	Dissection of wall beginning far down in right vertebral and continuing throughout basilar artery, occluding lumen; no mention of changes in brain
Sudden onset of severe headache and left hemiplegia in patient with known neurosyphilis; hemiplegia nearly gone in 2 days; strabismus, left facial weakness and right hemiparesis developed 17 days later; death occurred suddenly from respiratory and cardiac failure	Short section of basilar artery showed dissection of media in 1/6 of circumference; severe arteriosclerosis of terminal part of internal carotids, but not of basilar or branches of circle of Willis; thrombus present in lumen of left vertebral and basilar arteries; condition of brain not stated
Headache and symptoms and signs suggesting brain tumor for 4 wk.; spinal Wassermann 4+; negative findings on trephining; postoperative purulent meningitis developed; death 12 days later	Spindle-shaped segment 18 mm. long of right vertebral artery showed dissection with occlusion of lumen; thrombus 10 mm. long in left vertebral and 7 mm. long thrombus in right internal carotid; no malacia of brain stem; purulent leptomeningitis present; syphilitic aortitis present
Automobile *vs.* bicycle accident; patient thrown into windshield; semicoma and flaccid right hemiplegia at once; cortical edema found on trephining; death in coma 2½ days after injury	Dissection of inner part of wall of terminal end of left internal carotid, 3 cm. of middle cerebral, and first part of anterior cerebral arteries with occlusion of lumens; diffuse softening of the left cerebral hemisphere; fractured rib; fat embolism demonstrated
Migraine attacks for years; had taken 10 grains thyroid extract daily for 3 yr. following thyroidectomy for Hashimoto's disease; sudden severe right facial and retro-ocular pain; later paresthesia and flaccid paralysis of left arm and leg; death in coma 3 days after onset	Dissection of inner part of media with occlusion of right middle cerebral artery for 2 cm., starting 1 cm. beyond origin and extending into first 2 branches for 1.5 and 2 cm. respectively; no arteriosclerosis; softening of right parietal and part of right frontal lobe; heart, 320 gm.

TABLE 1.—*Dissecting Aneurysms of Arteries Other*

Case	Author Year	Artery	Age	Sex	Blood Pressure	Days Duration	Extent of Dissection	Thrombosis	Infarction	Intimal Tear
16	Bigelow[10] 1955	Right middle cerebral	46	F	?	1	13 mm.	+	+	+
17	Watson[4] Case 4 1956	Basilar	32	M	130/90	4	2+	0	+	0
18	Norman and Urich[15] 1957	Right middle cerebral and major branches	15	M	?	14½ yr.	3+	0	+	+
19	Northcroft and Morgan[20] 1944	Left internal carotid in neck	31	M	?	2	3.8 cm.	+	+	+
20	Sirois, Lapointe, and Côté[21] 1954	Left common carotid	50	M	?	1	10 mm.	0	+?	+
21	de Grood[22] 1954	Right common carotid	50	M	125/80	3	7 cm.	0	?	0
22	Boyd and Watson[9] Case 1 1956	Right common carotid	42	M	?	3	2+	+	+	+
23	Austin and Schaefer[18] 1957	Innominate right and left common carotids	25	M	140/80	3	5 cm. 2 cm.	0	+	0
24	Crumpton[24] 1950	Pulmonary and branches	19	M	145/70	10 hr.	4+	0	0	+
25	Odinokova[25] 1956	Pulmonary proximal	43	F	115/80	2	2+	0	0	
26	Bauersfeld[14] 1947	Superior mesenteric	87	F	118/75	21?	5.5 cm.	+	+	+
27	Brewer[27] 1941	Left common iliac	50	M		?	4 cm.	0	0	+
28	Coenen[28] 1927	Right brachial Surgical specimen	51	M	180/95 L & R	15	4 cm.	+	+	+
29	Watson[4] Case 1 1956	Splenic	41	M	250/145	7 hr.	6 cm.	0	0	+
30	Garron[13] Case 1 1953	Central artery of retinal branch	74	M	?	?	2+	0	0	+?
31	Garron Case 2 1953	Central retinal artery and branches	66	M	?	?	3+	0	0	0

Clinical Summary	Anatomic Findings
Severe headache for 10 days; berry aneurysm of right middle cerebral artery found on angiogram; coma developed after air injection; berry aneurysm removed surgically; left hemiplegia developed; death in coma on day after surgery	Dissection of outer media for distance of 1.3 cm. beyond suture line in right middle cerebral artery with complete occlusion; recent thrombus in lumen beyond aneurysm; recent softening in portion of right cerebral hemisphere supplied by involved artery
Previous good health; onset of mental confusion, occipital headache, loss of vision, followed soon by semicoma; findings suggesting pontile hemorrhage; spinal fluid clear; death on 4th day, from respiratory failure	Dissecting hemorrhage in inner layers of distal end of basilar artery markedly narrowing lumen; softening present in ventral part of midbrain, upper pons, and hypothalamus; no cerebral arteriosclerosis; bronchopneumonia in lungs; other organs normal
Normal infant until 6 mo. old, when he had severe epileptic fit followed by permanent spastic left hemiplegia and lack of mental development (idiot); death at 15 yr. from pulmonary tuberculosis	Healed dissecting aneurysm (with functional channel) of right middle cerebral and major branches; extensive old softening of part of brain supplied by diseased artery; chronic tbc of left lung, pleura, and pericardium
Healthy soldier dragged by rope around neck; onset of coma and right hemiplegia on next day; death in coma 48 hr. after accident	Tear of intima and media with dissecting medial hemorrhage in proximal 3.8 cm. of internal carotid with occlusion of lumen; thrombus in lumen of distal portion and in middle and anterior cerebral branches; massive left cerebral softening; tear and hemorrhage in left sternocleidomastoid muscle
Increasing headaches, motor speech disturbance, weakness of right arm and leg; left common carotid injured by needle while injecting Diodrast for arteriography; death in coma next day	Large valve-like flap, 1 cm. long, produced by tear by needle, of inner layers of wall from outer part in left common carotid; lumen occluded by valve action; large left temporal spongioblastoma
Headaches, bizarre behavior for 4wk.; left facial paresis; Diodrast arteriogram showing complete obstruction of injected carotid; ventriculogram—pattern of right frontal tumor; resection of frontal lobe for glioblastoma; death in coma 3 days after resection	Injury of right common carotid by needle; dissection of wall by hemorrhage for distance of 7 cm.; blood drained from needle hole in intima; no tear; bronchopneumonia of right lung
Crushing injury by heavy machinery of chest and right side of neck; in few hours semicoma and left hemiparesis; temporal pulse absent; diagnosis common carotid thrombosis; death in coma on 3d day after injury	Tear of intima and media of right common carotid 1.5 cm. proximal to bifurcation; dissecting aneurysm in media proximal to tear; red thrombus in distal part, internal and external carotids, and anterior and middle cerebrals; ischemic softening of right cerebral hemisphere
Clinical case of Marfan's syndrome with typical arachnodactyly; sudden onset of neurologic symptoms with spasticity of all extremities and hyperactive reflexes; rapid central nervous system depression and death 3 days after onset	Dissection of media of terminal part of right innominate and adjacent third of right common carotid, occluding lumen; similar dissection 2 cm. long in left common carotid 3 cm. from origin; softening of entire right cerebral hemisphere; thickened wrinkled mitral leaflets; classic long extremities
Shepherd boy with cyanosis from birth due to congenital heart disease; sudden pain in chest, dyspnea, and increasing cyanosis; loud cardiac murmur; death 10 hr. after onset	Large dissecting aneurysm extending from origin of main pulmonary into terminal branches; circumferential tear in intima 1 cm. beyond origin of artery; external rupture with hemorrhage in mediastinum and into lungs; huge 36 oz. heart with common truncus arteriosus and large arteriovenous connection
Rheumatic heart disease from childhood; sudden onset of chest pain, radiating to left arm, weakness, dyspnea and cyanosis; clinically rheumatic heart disease with embolic cardiac infarction; sudden death 2 days after admission	Dissecting aneurysm of pulmonary artery (extent?) beginning 2.5 cm. above pulmonary valve; rupture of anterior lateral wall into pericardium producing cardiac tamponade; marked rheumatic mitral stenosis; heart weight, 240 gm.; right ventricle 8 mm. thick
Slight epigastric pain, nausea, and vomiting for 3 wk.; x-ray showed dilated loop of bowel consistent with clinical impression of partial bowel obstruction; death from same	Dissection of media for 5.5 cm. distal to tear, located 2.5 cm. from aorta; thrombus in lumen; gangrene of small bowel with adynamic intestinal obstruction
Severe pain in left lower abdomen for 1 mo.; dull pain radiating down left leg for days; sudden death; Negro patient	Dissecting aneurysm of proximal part of left common iliac with splitting of media for 4 cm., beginning in atheromatous ulcer; rupture into abdominal cavity; death from hemorrhage; marked arteriosclerosis of aorta
Trauma from crutches used 46 yr. following polio atrophy of left leg; gangrene of right hand and fingers developed in 15 days; segment of brachial artery removed and supplanted by piece of vein; gangrene progressed; amputation 4 days later; urine sugar, 1%	Arterial specimen 8 cm. long with central cylindrical swelling; intima torn from media; hemorrhage with clot between intima and media and in lumen in segment 4 cm. long; no change in arterial wall above or below central lesion
Fatigue and headaches for some months; sudden right hemiparesis and dysphasia; coma in few hours; hypertensive retinopathy; death 7 hr. after onset	Dissecting aneurysm 6 cm. long in proximal part of splenic artery with external rupture into pancreas; recent massive hemorrhage in left cerebral hemisphere, midbrain, and pons; malignant nephrosclerosis; heart, 490 gm.
Poor vision in right eye; detached retina found; melanoma suspected; eye removed surgically in 1946	Splitting of wall with dissecting hemorrhage in media of branch of central artery; rupture into adventitia; retina detached and elevated by bloody fluid; no tumor found
Blind painful eye following keratitis and corneal ulceration due to injury by foreign body 6 mo. previously; eye removed surgically in 1950	Intima and internal elastica separated from outer coat by hemorrhage in central retinal artery and branches; subacute keratitis and active iridocyclitis; retinal, preretinal, and subretinal hemorrhage; detachment of retina by hemorrhage

TABLE 2.—*Dissecting Aneurysms of Arteries Other Than Aorta: Authors' Cases*

Case	Artery, Year	Age	Sex	Blood Pressure Heart Weight	Duration, Days	Extent of Dissection	Thrombosis	Infarction	Intimal Tear	Aortic Arteriosclerosis	Clinical Summary	Anatomic Findings
1	Right renal 1943 (Fig. 1)	27	M	220/150 560	4	4.8 cm.	+	+	+	+	Severe headaches and progressive hypertension in last 2 yr.; left ureteronephrectomy for stones in distal ureter, hydronephrosis, and hydroureter, and marked atrophic pyelonephritis (Goldblatt kidney); in last 4 days decreasing output of urine and rise of NPN to 86 mg. %; death 13 days after operation	Dissection of intima and internal elastica from muscle by hemorrhage, plus thrombosis extending from a level 2 cm. from aorta to bifurcation of right renal artery; branches free; accessory artery to upper pole; extensive renal infarction; left kidney absent; hypertrophied heart
2	Right renal and superior mesenteric 1951	58	M	260/140 490	5	7 mm. 10 mm.	0 0	+ 0	0 0	2+	Hypertension known for 9 yr.; rising NPN in last 6 mo.; severe substernal pain 5 days before death; EKG, no infarct; complete anuria in last 4 days; uremia; muscular twitchings last 2 days; NPN, 108 mg. % and creatinine 6.8 mg. %; 4 days before death	Dissecting hemorrhage in outer media of right renal and superior mesenteric artery; thrombus in lumen of renal distal to dissection, and in upper branch of left renal artery; focal infarction in both kidneys; profound arteriolar nephrosclerosis; hemorrhage in mediastinum, origin not found; aorta free; hypertensive heart
3	Left renal, right renal branch, left common iliac 1952	79	M	170/100 520	2	2.5 3 4.5 cm.	0 0 0	0 0 0	0 0 +	3+	Carcinoma of prostate with bone metastases known for 2 yr.; anterior myocardial infarct in 1950; progressive anemia and signs of cardiac failure with angina in last few months; moderately severe epigastric pain in last few days; urinary output by catheter-850 cc. in last 48 hr.	Dissection of outer media of left renal for 2.5 cm., beginning 6 mm. beyond origin and terminal 3 cm. of lower branch of right renal artery; proximal 4.5 cm. of left common iliac narrowed markedly by hemorrhage in outer media; aorta free; marked coronary sclerosis; old and recent cardiac infarction with findings of failure; carcinoma of prostate with bone metastases
4	Major branches of right renal (2 of 3) 1954 (Fig. 2)	72	F	160/100 480	?	2.5 cm. 2.5 cm.	+	+	0	3+	Rheumatic aortic stenosis since childhood; moderate cardiac symptoms with auricular fibrillation in last few months; sudden syncope; left hemi-	Each of upper 2 main branches of right renal artery showed dissection of outer media for 2.5 cm.; lower branch free; extensive right renal infarction. Calcific

											24 hr.; death in coma 4 days after onset; urine protein 3+, many casts; NPN 56 mg. % day before death	ized arteriosclerosis, including cerebrals; fairly extensive softening in right cerebral hemisphere
5	Right renal 1957 (Fig. 3)	76	F	104/66 250	?	1.5 cm.	0	0	0 ?	2+	Hiatus hernia known for 11 yr.; emaciation from difficulty in swallowing for many months; surgical esophagoplasty for stricture and benign ulcer; poor response after surgery; continued low blood pressure; death on 9th day after operation	Incidental fresh hemorrhagic dissection of outer media for 1.5 cm. in right renal artery beginning 1 cm. from origin; no renal infarction; death from embolism of branches of both pulmonary arteries and postoperative gastrointestinal hemorrhage
6	Right renal and branches 1957 (Fig. 13)	52	M	220/150 610	? 10?	4.5 cm.	0	0	0	2+	Known to have had severe hypertension for months; cardiac failure in June and September, 1957; terminal admission in uremic coma; BUN 188 mg. %; severe retinopathy; edema of lower limbs; death in December, 1957	Incidental dissection of outer media of distal 2.5 cm. of right renal artery extending 2 cm. into each of two major branches; lumen moderately narrowed; no renal infarction; malignant nephrosclerosis; marked hypertrophy and dilatation of heart
7	Right renal and branches Left renal 1958	72	M	140/110 400	?	5 cm. 7 mm.	0 0	+ 0	0 0	2+	Fever for 10 days (42 C on admission); rales in lung bases; cyanosis; WBC 6,000; mental confusion; rapid decline in next days; clinically overwhelming bronchopneumonia	Incidental dissection of outer media of right renal, starting 1 cm. from aorta and extending 2 cm. into upper and middle branches; separate artery to lower pole free; recent 3 cm. infarct in midpart of right kidney; irregular intramural hemorrhages in segment 7 mm. long in left renal artery 1.5 cm. from origin; bilat. hemorrhagic bronchopneumonia
8	Left anterior descending coronary 1938 (Fig. 4)	63	M	240/130 850	5	8 mm.	0	+	+	3+	Chronic active glomerulonephritis, 5 yr. duration, with increasing hypertension; proteinuria, 3; in last 5 days severe dyspnea and cyanosis; no chest pain; markedly enlarged heart, systolic murmur, gallop rhythm; sudden death	Hemorrhage in outer zone of media of midpart of anterior descending coronary artery in segment 8 mm. long; complete occlusion; marked hypertrophy of heart; large anterior and apical infarct with rupture and hemopericardium (700 cc.); chronic glomerulonephritis
9	Major branch of superior mesenteric 1947 (Figs. 5 & 11)	75	F	110/70 250	7	4 cm.	0	+	+	2+	Sudden onset and rapid progression of symptoms of intestinal obstruction, cause unknown: abdominal pain, distention, fecal vomiting; hospital entry delayed; patient too ill for surgery; death on 7th day	Dissecting hemorrhage in outer media in first 4 cm. of major branch of superior mesenteric artery; marked occlusion; main trunk free; extensive infarction of 75 cm. of small intestine, beginning at ligament of Treitz; distal artery and veins open

TABLE 2.—*Dissecting Aneurysms of Arteries Other Than Aorta: Authors' Cases*—Continued

Case	Artery, Year	Age	Sex	Blood Pressure Heart Weight	Duration, Days	Extent of Dissection	Thrombosis	Infarction	Intimal Tear	Aortic Arteriosclerosis	Clinical Summary	Anatomic Findings
10	Branch of superior mesenteric 1947	73	F	162/78 310	?	1 cm.	0	0	0	2+	Marked splenomegaly with leukemoid blood picture for 2 yr., clinically agnogenic myeloid hyperplasia of spleen; rapid terminal decline with fever (38-39 C) and ascites; no vascular symptoms	Incidental finding of recent dissection of outer media of 1 cm. of branch of superior mesenteric artery; no infarction of intestine; marked osteosclerosis and fibrosis of marrow; spleen, 1,540 gm.; tuberculosis of peritoneum, right pleura, abdominal and thoracic lymph nodes
11	Superior mesenteric and major branches 1948 (Fig. 6)	55	M	156/110 500	7	9 cm.	0	+	0	3+	Hypertensive heart patient developed severe dyspnea, cyanosis, and auricular fibrillation; sudden onset of abdominal pain and distention, followed by vomiting and lack of bowel movement; death 7 days after onset of abdominal symptoms	Dissection of outer media by hemorrhage, with occlusion of 9 cm. of superior mesenteric artery, beginning 6 cm. from aorta; extension into first part of major branches; marked gangrene of 40 cm. of midileum; hypertensive heart; generalized passive congestion
12	Celiac, splenic, hepatic, & branches left gastric 1951 (Fig. 7)	66	M	250/140 450	?	3+ 5 cm. 3 3 2 cm.	0	0	6 0 0 0 0	2+	Severe hypertension for years; dizziness and headaches in last months; partial hemiparesis, restlessness, twitching in last 2 wk.; severe cerebrovascular accident with sudden onset of coma and death in 4 hr.; no abdominal symptoms	Recent dissection in outer media of celica axis beginning 1 cm. beyond origin, extending through common hepatic, into liver in left hepatic and first part of right hepatic, splenic, and left gastric arteries; massive left cerebral hemorrhage with rupture into lateral ventricle; cardiac hypertrophy
13	Common hepatic 1957	54	M	300/140 650	?	1.5 cm.	0	0	0	+	Marked hypertension known for months; sudden severe pain in chest and upper abdomen; heart enlarged; gradual temporary subsidence of pain; sudden death after convulsion	Incidental dissecting hemorrhage of 1.5 cm. of common hepatic artery, independent of aorta; celiac and proximal part of hepatic free; separate dissecting aneurysm of aorta from arch to bifurcation; rupture into left side of thorax; marked cerebral sclerosis; brain negative; marked arteriolar nephrosclerosis
14	Pulmonary main and right	26	F	110/70	½ hr.	12.5 cm.	0	0	+	0	Known case of congenital	Extensive dissecting hemorrhage

	1947 (Figs. 9 & 10)			300						heart disease, markedly handicapped since birth, possibly patent ductus; sudden onset of severe substernal pain, shock; coma, and death within a half-hour	in main pulmonary and first 6 cm. of right branch. Long intimal tear. Rupture externally into mediastinum. Aorta free. Patent ductus arteriosus, marked dilatation and atherosclerosis in pulmonary artery.
15	Branch of right pulmonary 1947	70	M	? 350	5?	1 cm.	0	0	0	2+	Bilateral slowly progressive pulmonary tuberculosis; fluid present in left chest; increasing dyspnea and cyanosis in last few weeks	Dissection of outer media of 1 cm. segment of secondary branch of pulmonary artery to right upper lobe; bilateral active fibrosing tuberculosis, especially in apices; cavity in right apex; effusion (2,500 cc.) in left chest; emphysema; ectasia of pulmonary arterial tree; bronchopneumonia of right lower lobe
16	Left common carotid 1950 (Fig. 8)	54	F	205/110 400	1	8 cm.	0	+	0	2+	Hypertension known for 5 yr.; recent cerebral symptoms thought due to possible tumor; poor filling of cerebral arteries shown by arteriogram made at 10 a.m. by puncture of midpart of left common carotid; coma developing late same day; death 7:30 next morning	Marked dissection by hemorrhage in outer media of entire length of left common carotid artery above and below needle puncture; complete closure in midpart; hemorrhage in carotid sheath; marked sclerosis of carotids and cerebral arteries; early extensive softening of left cerebral hemisphere; heart enlarged
17	Left superior thyroid and 2 branches 1954	55	M	280/160 830	?	1 cm. 1 cm.	0	0	0	2+	Known severe hypertension for 6 yr., average B.P. 240/120; severe headaches began 14 wk. before death; increasing azotemia in last 6 wk.; death in uremic coma	Dissection of outer media of distal 1 cm. of left superior thyroid artery, extending 1 cm. into each of two major branches (incidental finding); marked cardiac hypertrophy; malignant nephrosclerosis; generalized arteriolar sclerosis 3 +

us clinical records, autopsy protocols, and tissues or slides for microscopic study in six other cases of peripheral arteries, which are included with our own cases in Table 2.

Observations

Arteries Involved.—Study of Tables 1 and 2 shows, in a total of 48 patients, the number of times in which the following arteries were involved: renal, one or both, or major branches, 12; coronary, 6; intracranial, including branches, 8; common carotid, including innominate, 5; internal carotid in neck, 1; superior mesenteric or major branch, 5; other abdominal, 3; pulmonary or branch, 4; brachial, 1; common iliac, 2; superior thyroid 1; retinal, 2.

Age, Sex, Race.—A glance will show that of the 31 cases reviewed in Table 1, 19 patients were less than 50 years old, and all but 5 were 53 years of age or younger. In our own series, there were only two younger than 52 years old, and 6 were between 70 and 79 years of age. Some of the disparity of age incidence between the two series can be explained on the basis of trauma. The nine patients in Table 1 whose lesion followed trauma include largely younger persons, while only one of our patients (Case 1) was a young person developing a dissection following injury. There were 34 male and 14 female patients in the combined series. Brewer's [27] patient (Case 27, Table 1) was a Negro, as was our Case 6. Our Case 13 was a Mexican. All the remainder in both groups were Caucasian.

Blood Pressure.—Blood pressure readings were recorded in 15 of the reported cases, and in only 5 of these was there elevation of systolic pressure above 150 mm. Hg. However, some of these patients were essentially healthy prior to injury, and the dissection of the involved artery resulted from trauma. High blood pressure readings, some exceptionally high, were present before or after hospital admission in 12 of the 16 of our own cases in which records are available. Eight of our patients had systolic pressures of 205 to 300 mm. Marked arteriolar nephrosclerosis was present in four cases and chronic glomerulonephritis in one case, and in Case 1 a hydronephrotic pyelonephritic contracted kidney had been removed 13 days before death. The weights of the hearts in both series corresponded well with the blood pressure readings. We may assume that there was pulmonary hypertension from the clinical and pathologic findings in the four pulmonary artery cases included in both series. None of these showed elevation of brachial artery pressure.

Watson [4] believes that acute reflex elevation of blood pressure due to cerebral hemorrhage may initiate hemorrhage and dissection in an artery already weakened by medial degenerative changes. In two of his four cases cerebral hemorrhage had occurred. In one of these cerebral cases (No. 29, Table 1) there was a dissecting aneurysm of the splenic artery and the blood pressure was 250/145 mm., even though the patient was comatose. There was hypertrophy of the left ventricle, and the heart weighed 490 gm. In his other case (No. 3) there is no mention of blood pressure, but the heart weighed 450 gm. and the left ventricular wall was thickened. In only one of our patients (Case 12) was there cerebral hemorrhage, and he also had been markedly hypertensive for years. Dissecting aneurysm of the celiac axis and its branches (Fig. 7) was an unsuspected autopsy finding.

Trauma.—Direct traumatic injury of the involved artery obviously played a major role in producing a dissecting aneurysm in nine of the cases already reported and in two of our own patients. One of Boyd and Watson's [8] cases (No. 5) showed dissection in the right renal artery, which was traumatized in separating it surgically from its bed overlying a massive retroperitoneal ganglioneuroma. It is possible that the right renal artery of our Case 1 was damaged by tension on the aorta, transmitted to the first portion of the right renal artery

during removal of the left kidney for stones and atrophic pyelonephritis. Hedinger's patient[9] (Case 9) was kicked by a horse, fracturing the sternum and injuring the heart and the left descending coronary artery, in which there developed a dissecting aneurysm 5 mm. long, followed by infarction and rupture of the left ventricle. Apparently, tension on the right middle cerebral artery during removal of a berry aneurysm injured the wall sufficiently, in Bigelow's[18] case (No. 16), to result in a dissection 13 mm. long, causing encephalomalacia.

Injury of the internal carotid artery in the neck by a loop of rope around the neck, in Northcroft and Morgan's[20] case (No. 19), caused a long medial hemorrhage. Direct damage to the head by being thrown against the windshield, in a bicycle *vs.* automobile accident, in Dratz and Woodhall's patient[16] (Case 14), led to a dissecting aneurysm of the internal carotid and middle and anterior cerebral branches. Damage to the common carotid artery, with splitting of the media and dissection by hemorrhage, during or following injection of opaque material for cerebral angiography, occurred in our Case 16, Table 2, and in the cases reported by Sirois et al.[21] (Case 20) and by de Grood[22] (Case 21). Sirois' patient apparently turned his head when the needle had traversed the artery, resulting in a clean cut through the intima and inner media and a tear in a segment of the media. A valve-like flap, thus produced, filled like a sail in the wind, occluding the lumen and resulting in encephalomalacia. De Grood's case is similar to our Case 16 in that both showed dissecting hemorrhage in the media for a long distance after traumatization by the needle. Boyd and Watson's patient (Case 22) developed his lesion in the common carotid artery after a crushing injury to the neck by heavy machinery.

Coenen's[28] patient (Case 28) is the only one in whom chronic trauma appears to have played a role. His patient, in whom the brachial artery was involved, used crutches for years, following atrophy and dysfunction of the left leg due to poliomyelitis in childhood.

Renal Arteries.—The renal artery, or one or more of its major branches, is the most commonly affected artery. The right renal artery or its branches were involved in seven cases and the left renal artery and branches in one case, and bilateral involvement of the main trunk or branches was found in four cases. As a rule a long segment of the artery was dissected, and frequently extension into branches, sometimes far out in the renal parenchyma, was observed. Varying degrees of narrowing

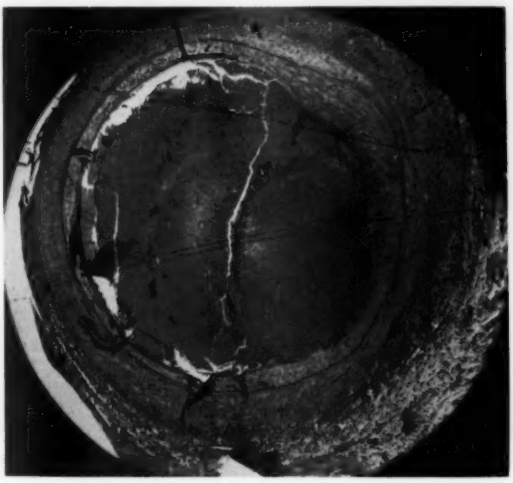

Fig. 1 (Case 1).—Right renal artery. Intima and internal elastica elevated from muscle by hemorrhage. Hemorrhage and thrombus occlude lumen. Verhoeff elastic tissue stain; × 14.

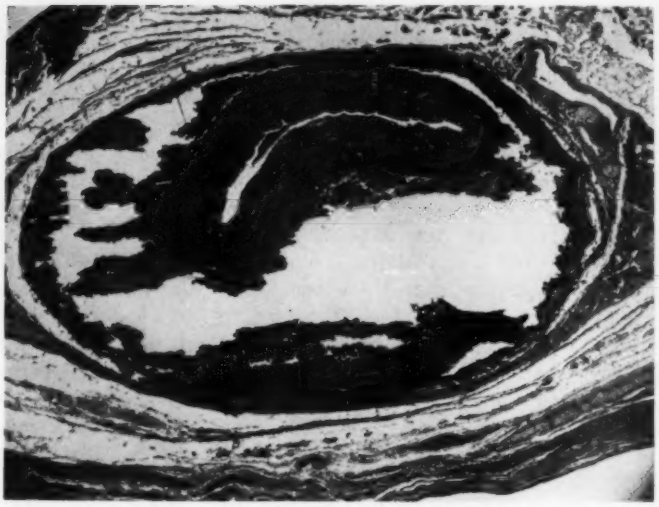

Fig. 2 (Case 4).—Major branch of right renal artery. Dissection between media proper and external elastic layer around entire circumference. Lumen closed. Clot has in part fallen out in preparing section. Hematoxylin-eosin stain; reduced to 88% of mag. × 30.

(Fig. 2), usually marked, was noted, and in 8 of 12 cases infarction of renal parenchyma was present. In six of the cases renal damage from circulatory embarrassment appeared sufficient to be the major cause of death. This is particularly true in our Case 1 (Fig. 1), in which only the right kidney remained after left nephrectomy for hydronephrosis and atrophic pyelonephritis. In three of our patients (Cases 2, 6, and 7) there was fairly marked stenosis of the orifice of the renal artery in a short segment by arteriosclerotic intimal thickening, and the dissecting aneurysm arose at various distances distal to the stenosed portion. The patient of Gilfillan et al.[7] (Case 2) was successfully operated upon 12 days after onset, and the involved renal artery and partially infarcted kidney were removed. He was still healthy after four years.

Coronary Artery.—Six cases, including one of our own (Case 8), have shown involvement of a coronary artery—always the

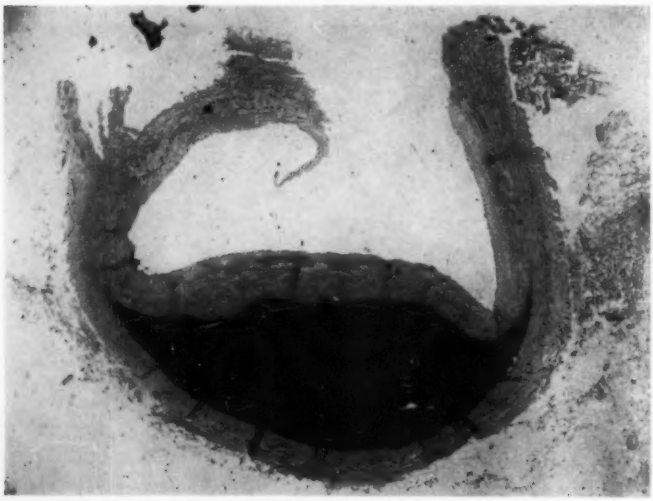

Fig. 3 (Case 5).—Right renal artery. Dissecting hemorrhage near middle of media. Artery opened by prosector. Hematoxylin-eosin stain; reduced to 88% of mag. × 17.

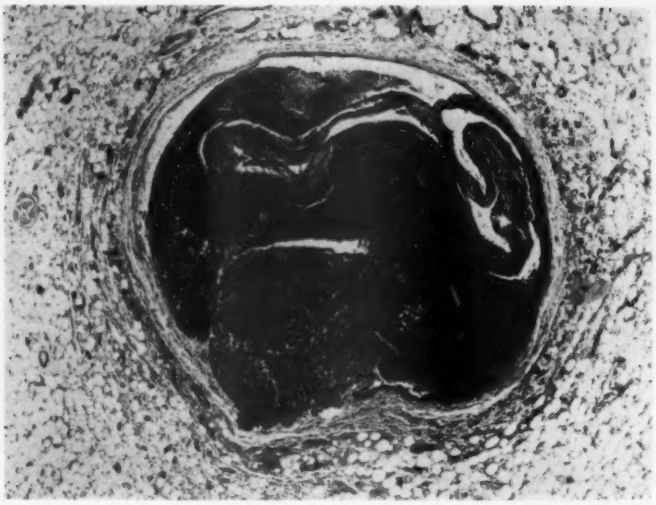

Fig. 4 (Case 8).—Left anterior descending coronary artery. Dissecting hemorrhage extending around nearly entire circumference. Complete closure of lumen. Elastica stain; reduced to 88% of mag. × 30.

left anterior descending artery. In three patients the lesion began at a level midway to the apex. In all six cases the clinical pattern and anatomic sequelae were similar to coronary thrombosis occurring in sclerotic arteries, and death resulted from the lesion. The first of these reported (Case 6, Table 1) was that of Glendy, Castleman, and White,[9] in 1937, although the patient died in 1902. The first case seen by us of dissecting aneurysm primary in any artery other than the aorta (Case 8) occurred in 1938 and involved a segment of the left anterior descending artery only 8 mm. long, midway to the apex. There was complete occlusion of the artery (Fig. 4), anterior and septal myocardial infarction, and cardiac rupture five days after onset. Ventricular rupture occurred also in Hedinger's Case 9. In contrast to these cases with involvement of only short segments is the case (No. 10) of Lovitt and Corzine,[12] in which there was dissection of the entire length of the anterior descending artery. This occurred in a healthy woman of 39, two weeks after normal delivery of a full-term, healthy child. Death occurred two and a half hours after onset of symptoms. Uehlinger's [10] two patients (Cases 7 and 8) were two middle-aged reserve Swiss soldiers who developed symptoms of coronary occlusion following strenuous military exercise and exposure to severe cold in the Alps in winter. Hedinger's [11] patient (Case 9) is the only one in whom direct trauma (a kick by a horse) played any role. In none of the coronary cases, including our own, was there any noteworthy degree of arteriosclerosis of the coronary arteries.

Intracranial Arteries.—We have not encountered any cases of dissection of intracranial arteries, but eight cases were reported in the literature (Cases 11 to 18 in Table 1). Clinical manifestations and outcome in these patients were similar to the effects of closure of these vessels by thrombi. The lesions were spontaneous in all cases exept two, due to trauma already mentioned (Cases 14 and 16 of Table 1). In all but Hyland's [14] Case 12 a long segment of the artery was involved. In Scholefield's [13] patient from Guy's Hospital in 1924 (Case 11), extensive mural dissection involved the right vertebral and basilar arteries, occluding the lumens as the hemorrhage spread along these arteries. Although a microscopic description is not given, the gross pattern is consistent with the diagnosis of dissecting aneurysm. Norman and Urich's [19] Case 18 is remarkable in that the lesion clinically

dated back to the age of 6 months, leaving the child permanently paralyzed and mentally retarded. Death occurred from intercurrent tuberculosis, at the age of 15. Organization and canalization, with formation of a double channel, such as one sees occasionally in the aorta, was present in the right middle cerebral artery and branches. Extensive old cerebral softening was found.

Carotid Arteries.—The common carotid has been the site of a dissecting aneurysm due to trauma in four cases, and the internal carotid in the neck, in one case. These have been discussed in a previous paragraph. In all these cases ischemic cerebral softening developed. The only nontraumatic case on record is the very unusual one of Austin and Schaefer [23] (Case 23), in which there was spontaneous dissection in the innominate artery extending to the right common carotid, completely closing the lumen of the latter. The left common carotid was partially occluded by a similar lesion. This case was clinically one of Marfan's syndrome with connective tissue alterations elsewhere.

Pulmonary Arteries.—Two cases involving the main pulmonary artery in the literature, and one (Case 14) in our series, were in young persons with cardiac lesions producing long-continued pulmonary hypertension. In our case a patent ductus arteriosus was present, and profound dilatation and arteriosclerosis of the pulmonary artery were found. A dissecting aneurysm extended from the main trunk into the right pulmonary artery (Fig. 9), and an intimal tear resembling one commonly seen in the aorta was found. Rupture externally, with bleeding into the mediastinum, was the immediate cause of death. This was also true in Crumpton's [24] Case 24, likewise secondary to congenital heart disease. Odinokova's [25] patient (Case 25) is the only one in whom mitral stenosis appears to have been the factor producing pulmonary hypertension. Rupture into the pericardial sac caused sudden death. In our second pulmonary case (Case 15), involvement of a branch of a pulmonary artery was an incidental finding in a patient dying of fibrosing chronic bilateral tuberculosis with emphysema.

Superior Mesenteric Artery.—Bauersfeld's [26] case (Case 26) is the only one in the literature in which the superior mesenteric artery was involved. The dissection of a segment 5.5 cm. long was accompanied by thrombus formation in the lumen. Death resulted from gangrene of the intestine due to ischemia. Our Cases 9 and 11 were free from thrombus formation (Figs. 5 and 6),

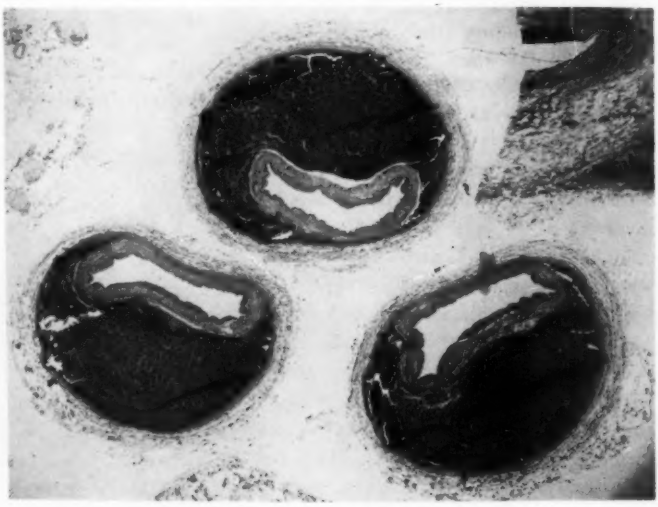

Fig. 5 (Case 9).—Major branch of superior mesenteric artery, multiple levels. Extensive hemorrhage between media and external elastic lamina. Marked narrowing of lumen. Elastica stain; reduced to 88% of mag. ×11.

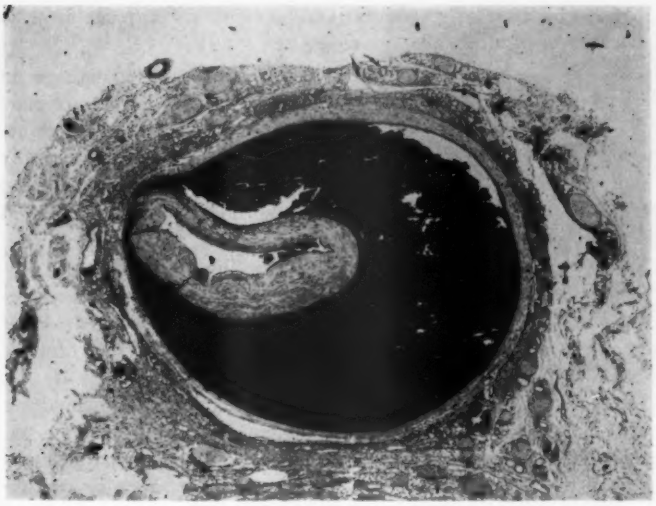

Fig. 6 (Case 11).—Main trunk of superior mesenteric artery. Complete circumferential dissection between media and external elastic lamina. Marked narrowing of lumen. Masson's trichrome stain; reduced to 88% of mag. × 15; green filter.

but in both the artery was narrowed markedly, causing infarction of a long segment of small intestine. A short segment of the main trunk of our Case 2 showed hemorrhage, without significant narrowing, and in Case 10 a branch was involved; but neither of these lesions was severe enough or of sufficient duration to produce gangrene of the bowel.

Other Arteries.—The common iliac artery in Brewer's[27] case (Case 27) showed dissecting hemorrhage in the media over a distance of 4 cm. and rupture anteriorly through the peritoneum into the abdominal cavity. The dissecting aneurysm of the splenic artery reported by Watson[4] and the aneurysms of the celiac, hepatic, and superior thyroid arteries in our series (Cases 12, 13, and 17) were incidental, unexpected findings at autopsy, and there was no evidence of disturbance of circulation in the tissues supplied by them.

Length of Lesion.—The length of the segment of artery involved by dissecting aneurysm has varied markedly. In the coronary case of Glendy et al. the lesion was only 4 mm. long, and in Uehlinger's and Hedinger's cases the involved segment was only 5 mm. long. Dissection by hemorrhage in our one coronary case (Case 8) extended throughout a portion 8 mm. long. Most of the renal artery lesions involved dissection of long segments. The most extensive involvement we have personally seen in any artery was in Case 12, in which the celiac axis and its branches were involved. Some branches of the left hepatic far into the liver showed medial dissection.

Arteriosclerosis.—Significant intimal changes of arteriosclerosis in the involved artery were uncommonly seen in our own material and in the cases already reported. One is struck by the absence of noteworthy intimal thickening or plaque formation in most of the photographs of arteries in the literature and in our own sections. The iliac artery of Brewer's 50-year-old Negro patient (Case 27) is described as having an atheromatous ulcer, from which point the aneurysm began. The aorta showed severe arteriosclerosis. In the 87-year-old patient of Bauersfeld[26] there was moderate atheromatosis of the superior mesenteric artery. In our own cases, there was moderate intimal thickening in the left renal and common iliac arteries of a 79-year-old minister (Case 3) and in the common carotid, which was injured during angiography, in Case 16; and there was most extreme intimal fibrosis, fatty plaque formation, and calcification in the pulmonary artery of Case 14, a young woman with a

patent ductus arteriosus. Moderate arteriosclerotic fibrous stenosis of the orifice of the renal arteries in Cases 2, 6, and 7 has already been mentioned. Beyond this level the intima was very little changed, except in Case 2, in which moderate intimal thickening was present. In our remaining patients intimal changes were mild or absent.

Fairly extensive changes of senile arteriosclerosis, moderate or severer, were found in the aorta of all but our youngest cases. The gross appearance of the aorta is described in only four of the cases reported in Table 1, and in only two of these was there extensive arteriosclerosis (Cases 1 and 27). Szabo's[15] case showed gross evidence of syphilitic aortitis, and during life a 4+ Wassermann test was present (Case 13).

Portion of Circumference Split.—Cleavage of the media involved a large portion of the circumference in nearly all of the arteries adequately studied and listed in Tables 1 and 2. In nine cases, of which five are our own, there was dissection of the entire circumference of the artery (Figs. 2 and 6). Of the remaining 32 cases there are only 3 in which less than 50% of the circumference was involved. Nine cases showed dissection halfway (Fig. 3), and twenty cases from 50% to 95%, around the artery.

Degree of Narrowing.—Hemorrhage in the wall had led to varying degrees of narrowing of the lumen of the affected arteries, except the pulmonary. In practically all other cases the lumen was reduced to less than half the normal diameter, and in most instances to less than 20%, owing to elevation of the inner coat of the aneurysmal sac and pushing of this inner layer toward the intima of the opposite side of the vessel (Figs. 2, 4, 6, 7). Our coronary case is a good example of this (Case 8). In some levels of this artery one could see no remaining lumen (Fig. 4). In the patients in whom a major pulmonary artery was involved, death from shock or rupture occurred before any effects of narrowing were manifested. Similar results are to be expected in dissecting aneurysms of the aorta.

Level of Dissection.—The level of the cleavage in the wall was most commonly in the outer portion. In 10 of the 25 cases in Table 1 adequately described in the literature, it was between the muscular portion of the media and the external elastica; in 8, in the media proper, usually in the outer portions, and in 7 cases the internal

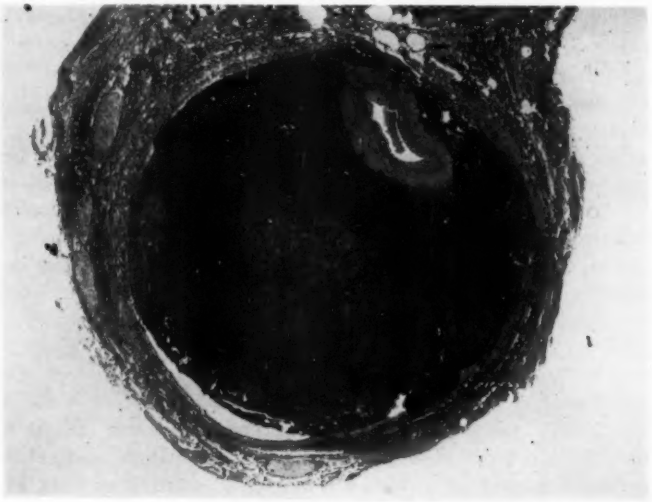

Fig. 7 (Case 12).—Common hepatic artery. Massive recent dissection and hemorrhage between media and external elastic lamina. Lumen nearly closed. Hematoxylin-eosin stain; × 12.

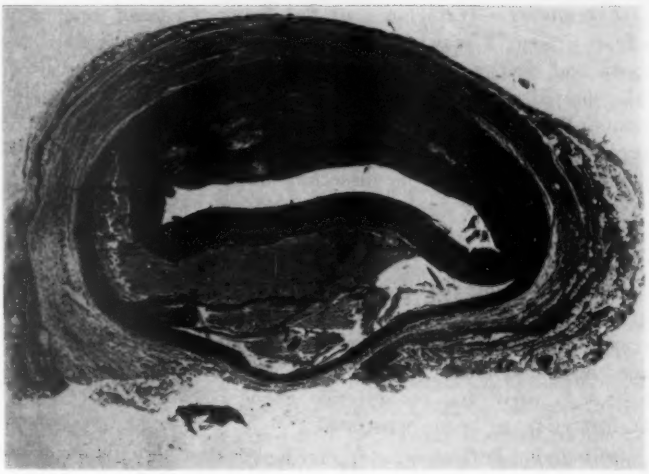

Fig. 8 (Case 16).—Left common carotid artery. Dissecting hemorrhage involving 50% of circumference in outer media, causing collapse of lumen. Marked intimal thickening in upper half of picture. Elastica stain; reduced to 88% of mag. × 11.

elastica was elevated from the muscle of the media. One of the last group was Coenen's case, with the brachial artery involved. In Garron's[29] two retinal artery cases the cleavage was apparently at this level, and in the four others (Cases 13, 14, 15, and 17) the intracranial arteries were involved. In all four coronary cases (Cases 7, 8, 9, and 10) the cleavage was in the zone between the media and the external elastic layer, as in all but one (Case 6) of the renal artery cases. In our own series (Table 2), the renal artery of Case 1 was the only one in which the internal elastic layer was lifted up from the media (Fig. 1). There were 6 cases of cleavage in the outer layers of the media and 10 cases in which the media was separated from the external elastica (Figs. 2, 4, 5). In the pulmonary (Cases 14 and 15, Fig. 9), the common iliac (Case 3), and the common carotid (Case 16, Fig. 8) arteries the dissection was in the outer part of the media proper, rather than just internal to the external elastica. All three of these arteries are elastic arteries, like the aorta, rather than muscular arteries, and the plane of dissection is similar to that seen in the aorta. The other three arteries in which the muscle of the media proper was split were the right renal and superior mesenteric arteries in Case 2, and the renal in Cases 3 and 5 (Fig. 3).

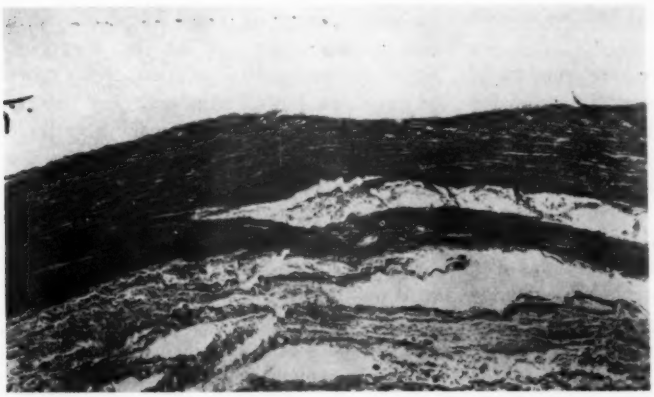

Fig. 9 (Case 14).—Right pulmonary artery. V-shaped split in outer media due to dissecting aneurysm. Blood has dropped out in handling. Hematoxylin-eosin stain; reduced to 88% of mag. × 20.

Thrombosis.—Thrombosis in the lumen of an artery in which a dissecting aneurysm had developed occurred in 9 of the 31 reported cases and in 2 of our 17 patients. In 6 of these 11 cases the thrombus occurred distal to the portion of the artery narrowed or torn by the dissecting aneurysm. In the other five cases the thrombus involved the portion in which separation of the coats had occurred. In 10 of these patients infarction of the organ supplied by the artery was present. In Uehlinger's remaining case (Case 7), death occurred one and a half hours after onset of symptoms, too early for recognizable changes of infarction to be demonstrated.

Infarction.—Infarction, of varying degree, was also encountered in many cases in which no thrombosis was present, and depended largely on the degree of narrowing and the length of time between the onset of the aneurysm and death. Infarction was present in 10 cases, absent in 9 cases (including 2 pulmonary and 2 retinal artery cases) and was not mentioned in 3 cases of the 22 summarized in Table 1. Of 15 thrombus-free cases of our own series, 6 showed infarction, and 9 (including 2 pulmonary and 1 thyroid artery case) did not.

The dissecting aneurysms appear to have played a major role in causing death in 23 of the 31 reported cases and in 8 of our 17 cases. Obviously, in some of our patients the lesion was a terminal event in the course of much more serious disease in other organs.

Intimal Rupture.—A tear or rupture of the inner wall of the aneurysm, allowing communication with the lumen, was demonstrated in 13 of the 31 cases in the literature. In six the rupture was found on gross inspection and in seven by microscopic study. We found such a defect in 5 of our 17 cases, in 2 of which it was seen grossly: pulmonary in Case 14 and common iliac in Case 3. However, these figures may not tell the true incidence of a breach in the continuity of the lining, since most of the arteries examined by others, as well as by us, were fixed before sectioning and then cut at intervals transversely. Most of us have made, from the external appearance of an involved artery, the gross diagnosis of thrombosis in the lumen and have placed the vessels in fixative for later transverse sectioning. Study of the intima of an artery opened longitudinally was made in only seven cases in the literature, and in seven arteries in five cases of our town. We found no rupture of the intima in four arteries so opened: the superior mesenteric and renal arteries in Case 2, and the renal artery in Cases 3 and 5. The thin lining of the dissecting aneurysm of the renal artery in our Cases 6 and 7 was torn by the dissecting scissors on the first cut (made on the wrong side) of the artery by the prosectors, who encountered this lesion for the first time, and we cannot say whether there was a tear or not. For smaller arteries, we feel that, on the whole, opening the vessels longitudinally will do more harm than good as far as completeness of study is concerned, and will ruin chances of preparation of good sections for microscopic study. Even in larger arteries, like the pulmonary, carotid, or iliac, great care must be taken to cut in the proper zone and to leave intact the blood-containing part of the artery involved by the aneurysm.

We serially sectioned with the microtome the lesion-bearing portion, which was only 8 mm. long, in the anterior descending coronary artery of Case 8 and found a tear in the inner coat of the aneurysm. Serial sectioning was not done in any other of our cases, but all were examined grossly and microscopically, after making multiple cuts transversely at close intervals. In the cases reported in Table 1, only two arteries were serially sectioned, the coronary in Uehlinger's Case 7 and in Lovitt and Corzine's Case 10. No breach in the inner coat was found in the short segment involved in Uehlinger's case. In Lovitt and Corzine's case, in which the artery was involved throughout its entire length, the

media was torn completely, but the internal elastic lamina had not given way.

According to Hirst,[1] 37 cases of dissecting aneurysm of the aorta without intimal tears have been reported in the English literature between 1933 and 1954. Of these, 21 were included in the 505 cases with individualized case reports. There is, therefore, abundant evidence in studies of dissecting aneurysms of the aorta, as well as of smaller arteries, that the lesion may begin as a hemorrhage in the media of an artery and the blood remain confined to this layer between the internal and the external elastic lamina, without a tear of the intima.

Microscopic Changes in the Media.—Much has been written about the pathologic changes in the media of the aorta in cases of dissecting aneurysms. Most of us have encountered in varying degrees the findings in the aorta described so clearly by many observers, particularly Erdheim,[31,32] Moritz,[33] Shennan,[3] and Sailer,[34] and, in more recent times, by Gore and Seiwert[35] and Gore.[36] The latter authors were able to demonstrate a degenerative process in the media of all the aortas of a large series of dissecting aneurysms studied at the Armed Forces Institute of Pathology. In younger persons, the elastic laminae were affected primarily, and there was destruction and loss of elastic tissue in varying-sized foci. Basophilic staining material, which is commonly referred to as mucin or mucoid material, but which is actually myxomatous tissue, was frequently abundant in cases in which there was loss of elastic tissue. Accumulation of this material in cyst-like pockets was particularly stressed by Erdheim. In older age groups, Gore and Seiwert found that the changes were predominantly in smooth muscle, which showed foci of necrobiotic degenerative changes, with even complete loss of groups of muscle fibers, accompanied by condensation of elastica, due to falling together of elastic lamellae. Admixtures of muscle and elastic tissue changes were commonly seen. It would be enlightening to know what one would find in the aortas of a large control group in which no dissecting aneurysms occurred, if they were studied with the same care as the above authors have used.

At first thought, one might presume that the pathologic changes in the media of peripheral or pulmonary arteries involved by primary dissecting aneurysm would resemble in appearance and frequency those seen in similar disease in the aorta. This is not necessarily true. In the first place, one must remember that most of the aneurysms reported by others and by us occurred in "muscular" arteries, which have an architecture different from that of elastic arteries, in which elastic laminae, muscle fibers, and ground substance are arranged in a pattern fairly similar to that encountered in the aorta. Arteries of the elastic type include the pulmonary, innominate, common carotid, subclavian, vertebral, and common iliac. According to Bailey, and to our own observations, as these arteries branch and become smaller, they gradually assume the structure of so-called "medium-sized," or "muscular" arteries.

One must also keep in mind that many of the changes seen in the wall of the involved artery may be due to disturbance of blood supply and nutrition of both the outer and the inner coat caused by splitting of the wall by dissecting hemorrhage, usually in a large portion of the circumference of the vessel. Shearing off of the vasa vasorium must occur in the outer part of the media, since in nearly all the muscular arteries the dissection was between the external elastic layer and the muscle of the media, and in nearly all of the elastic arteries the dissection was in the outer part of the media. Degenerative changes, and even necrosis, of smooth muscle are to be expected if the lesion is of sufficient duration to allow effects of lack of blood supply to occur. Edema and leukocytic infiltration secondary to the injury of the wall, occurring especially adjacent to the line of the

tear, further complicate the histologic pattern. Lack of blood flow through the lumen and pressure effects due to the extravasation of blood in the wall may well interfere with nourishment of the inner coat through the intima. Likewise, changes secondary to the obstruction of blood flow may occur in portions of the arteries distal to the aneurysm, or possibly even proximal to the occlusion.

The changes in the media resulting from arteriosclerosis and the aging process, or those subsequent to long-continued hypertension, must be considered and ruled out before one assumes that any alteration in the histologic pattern is specific for dissecting aneurysm. Postmortem effects such as separation and elevation of the internal elastic lamina and degenerative changes in smooth muscle, are sometimes difficult to interpret. Also, one wonders whether the pattern of the elastica in the media may not be altered by vigorous stretching or pulling on an artery, such as the renal, as the kidney and its vessels are removed by clumsy technique.

When all of these factors are considered, it becomes obvious that one can hardly expect to encounter uniform microscopic findings in arteries of various sizes, types, and locations in a series of dissecting aneurysms. Only seven of the recorded cases in Table 1 showed involvement of an "elastic" artery, viz.: pulmonary, two cases; common carotid alone, three cases; innominate and both common carotids, one case, and common iliac, one case. Crumpton did not record the microscopic findings in her pulmonary case. Odinokova interpreted changes in the media of her patient as being rheumatic in origin. Active rheumatic valvular changes were present in the heart. Of the carotid cases of Sirois et al., de Grood, Northcroft, and Morgan, and Boyd and Watson, all four of which were traumatic in origin, only the last two were described microscopically, and no changes were reported. Extensive fragmentation and disorderly arrangement of elastic fibers and the presence of numerous small cystic spaces, filled with slightly basophilic and Schiff-positive substance, were present in the right common carotid artery and aorta in a patient with Marfan's syndrome reported by Austin and Shaefer (Case 23). This man had marked changes in connective tissue in the endocardium and other parts of the body.

No mention of microscopic medial changes is made in 7 of the 23 muscular arteries described in the literature. In 5 cases microscopic study revealed no changes, and in the remaining 11 cases some degree of deviation from normal was reported. In the renal artery Liebow et al.[6] (Case 1) found "medial degeneration involving smooth muscle. The elastic tissue was uninvolved." "Some medial degenerative changes" are reported by Gilfillan et al. (Case 2) in their renal artery case. Degeneration and necrosis of muscle at the border of the aneurysmal sac and patches of mucoid degeneration in the media were observed by Klotz in his study of the basilar artery of Hyland's patient (Case 12). Szabo interpreted the changes in the vertebral artery in his case (No. 13) as being syphilitic; but, from his description in the absence of illustrations, it appears to us that the findings are better explained on the basis of partial healing, with production of granulation tissue in the edges of the defect made by dissection fully 40 days before death. Lack of muscle fibers, possibly largely due to the normal decrease in small arteries, and possibly partly due to sclerotic changes, was present in the central arteries of the retina of the two surgically removed eyes studied by Garron (Cases 30 and 31).

More extensive changes are reported in the coronary arteries of Lovitt and Corzine's 39-year-old patient (Case 10), in whom much intimal thickening due to fibrous tissue and fatty deposit was present. In addition, irregular myxomatous replacement of individual muscle cells or groups of muscle cells was present in the widely dissected left descending, as well as in the other coronary arteries. Many adjacent

muscle cells showed homogeneous cytoplasm with loss of fibrillae. Diffuse inflammatory response to the hemorrhage with leukocytic infiltration was seen in the periadventitial fat. Watson found in all four of his cases (splenic, renal, renal branches, and basilar) "evidence of medial degeneration, manifested by degeneration and loss of smooth muscle cells, by irregularity in the arrangement of elastic fibers and by formation of a fibrillary connective tissue, containing variable amounts of metachromatic ground substance and sparsely distributed stellate cells."

The original sections of the arteries of our own cases were reviewed, and new sections, when needed, were studied after use of various stains, including Verhoeff's elastica stain, Alcian blue and chromatrope, Hotchkiss and McManus' PAS, and Alcian blue and PAS, and others with aniline blue or toluidine blue. In most instances we had available for study levels of arteries above and below the aneurysm, as well as multiple sections through the dissected portions. Arteries from other parts of the body were also sectioned in some cases, and in half of the cases the aorta was studied microscopically. We were quite impressed with the changes secondary to the splitting of the wall in most of the arteries of these patients. Commonly, if over a day had elapsed after the development of the aneurysm, necrosis of muscle at the margins of the defect was conspicuous. In some instances leukocytic infiltrate was seen, especially in the outer layers. Also, edema was often present in the media, sometimes to a fairly marked degree, even in portions of the circumference which were not split. Fluid from this source separated muscle fibers and produced a pattern superficially resembling an increase of mucoid material.

Changes obviously not secondary to the dissection were present above and below, as well as in the involved segment, in both elastic and muscular arteries. Increase in interstitial tissue, or so-called ground substance, replacing small groups of muscle or separating muscle fibers, was encountered in varying degrees in all cases, sometimes rather marked. Portions of this tissue were distinctly fibrillar, and parts were more or less homogeneous; but both components stained deeply and conspicuously with Alcian blue or aniline blue. The pulmonary artery (Fig. 10) of Case 14 showed the severest alteration of all. Much of the wall showed heavy deposit of in-

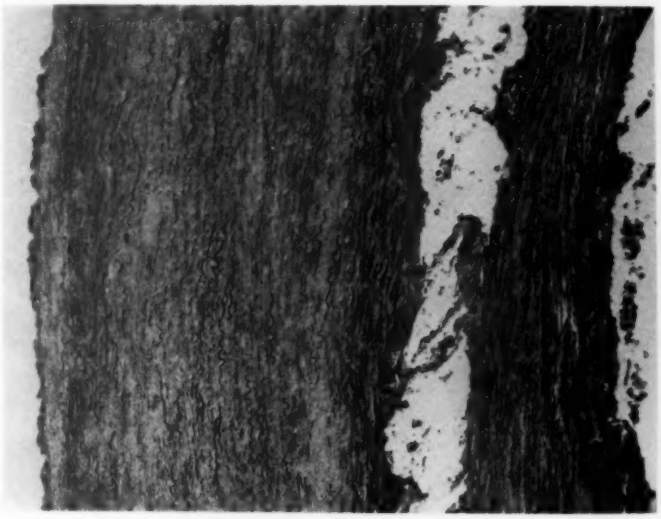

Fig. 10 (Case 14).—Right pulmonary artery. Split present deep in media on right. Internal portion of media shows fragmentation and loss of elastic tissue and muscle and marked increase in ground substance. Alcian blue and chromatrope; reduced to 88% of mag. × 65.

Fig. 11 (Case 9).—Branch of superior mesenteric artery distal to dissected portion. Irregular loss of muscle and replacement by homogeneous ground substance. Alcian blue and chromatrope; reduced to 88% of mag. × 100.

creased ground substance in areas, or sheets of varying size and shape. Widespread loss of muscle fibers and elastic tissue was present. Much less change was present in the other two elastic arteries—the common iliac in Case 3 and the common carotid in Case 16. These showed little alteration of elastica, but the chief change was the irregularly distributed increased ground substance.

The remaining arteries were of the muscular type. Loss of muscle in irregular foci and replacement by homogeneous or finely fibrillar matrix were seen in all to some degree, but most marked in the superior mesenteric artery (Fig. 11) of Case 9. Elastic tissue changes varied considerably, also. Fragmentation and loss of elastic fibers in portions showing the most increase in interstitial tissue were conspicuous. In many there was formation of skeins (Fig. 13) or irregular aggregates of fibers, as though they had broken loose from their anchoring foci and had contracted into

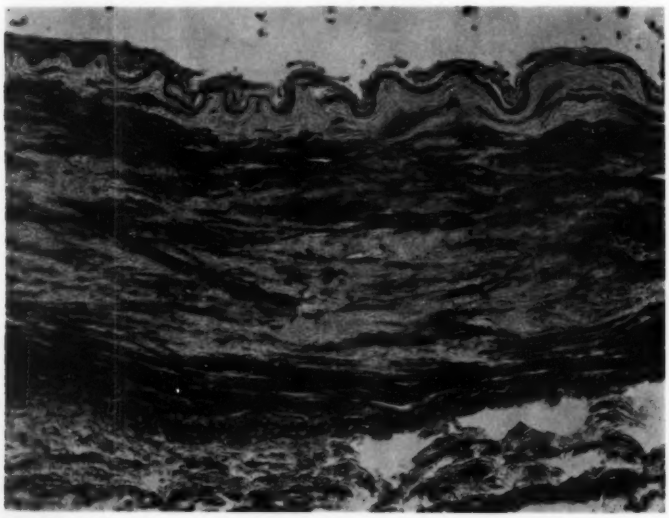

Fig. 12 (Control).—Renal artery of 81-year-old woman who died of lymphosarcoma. No hypertension. Marked irregular loss of muscle and increased ground substance. Alcian blue and chromatrope; reduced to 88% of mag. × 125.

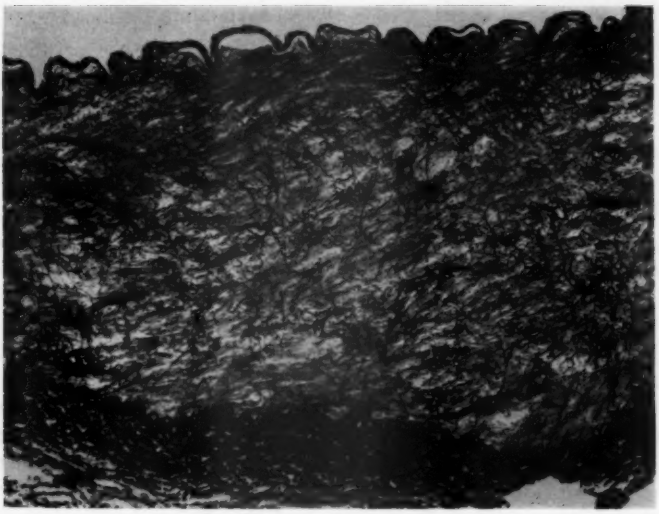

Fig. 13 (Case 6).—Right renal artery. Irregular fragmentation and skein formation of elastic fibers. Elastica stain; reduced to 88% of mag. × 125.

tangled webs. Similarly disorganized elastic tissue was described by Watson in his four cases.

In none of the arteries was the ground substance stainable by PAS, either when used alone or in conjunction with Alcian blue. One could see in about half of the cases PAS-positive material in minute amounts, not in the ground substance itself but apparently in fusiform cells, possibly smooth muscle cells or fibroblasts. This material appeared in small globules, reminiscent of fat in a necrosing cell. We found no cyst-like accumulation of "mucus" in any of the involved arteries or in the aortas of eight of our patients.

A series of 15 control arteries from routine autopsies, including elastic and muscular peripheral arteries, was obtained from six patients, of ages 56 to 81. These were stained, as were the arteries listed in Table 2. In all of them there were increase

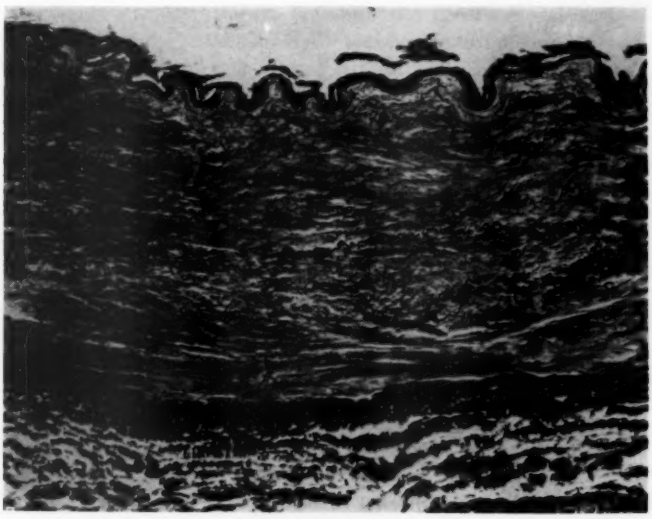

Fig. 14 (Control).—Renal artery. Same case as Figure 12. Irregular fragmentation and skein formation of elastic fibers. Elastica stain; reduced to 88% of mag. × 100.

in ground substance, focal loss of muscle (Fig. 12) or of elastic tissue, and, commonly, clumping of elastic fibers (Fig. 14), similar to that seen in our cases of dissecting aneurysm. We must come to the conclusion that we have not found evidence that there is a lesion which is specific for peripheral arteries subject to dissecting aneurysm.

Summary

Dissecting aneurysm of peripheral or pulmonary arteries occurs much less frequently than in the aorta, but 31 cases have been reported in the literature in the last 33 years. To these we add 17 cases in which one or more arteries were involved. The renal artery is the one most often affected, followed by the coronary and intracranial arteries.

Hypertension was present in 12 of our 17 cases, but in only a third of the cases reported in the literature in which blood pressure readings were given. Trauma apparently initiated the lesion in 11 of the total 48 cases. Three of these developed after needle puncture of the common carotid for angiography.

Narrowing of the lumen with infarction of the organ supplied by the artery was the rule in peripheral arteries. Three dissecting aneurysms of the pulmonary artery, terminating by rupture, occurred in cardiac cases—two congenital and one of rheumatic mitral stenosis. A tear in the intima was demonstrated in 18 of the 48 cases.

The dissecting aneurysms appear to have played a major role in producing death in 31 of 48 cases.

A pathologic change in the media specific for dissecting aneurysm was not demonstrated in our material.

The authors wish to thank Drs. Hugh Edmondson and Victor Gieshen, of the University of Southern California School of Medicine, for the contribution of Case 7, and the following members of the Department of Pathology of the College of Medical Evangelists for the following cases: Cases 5, 6, and 13, Dr. Albert E. Hirst Jr.; Case 11, Dr. Carrol Small; Case 17, Dr. R. H. Seasly Jr.

Huntington Memorial Hospital (2).

Addendum

Since this paper was read, two pertinent articles have appeared in recent literature. A case of dissecting aneurysm of the left coronary artery and the first portions of the descending and circumflex branches, with infarction of the anterolateral wall of the left ventricle and anterior half of the interventricular septum, was reported by Boschetti and Levine.[37] Death occurred three days after an acute onset of symptoms classic for coronary occlusion. Cystic medionecrosis was found in the involved arteries.

A good summary of traumatic changes observed in the carotid arteries following arteriography, including a case of dissecting aneurysm in a carotid, is given in an article by Fleming and Park.[38]

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Effect of Methylcholanthrene on the Respiratory Tract of the White Pekin Duck

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Studies have shown that methylcholanthrene in acetone applied locally to the skin of white Pekin ducks produces a variety of neoplasms, among which are papillomas, fibromas, neurofibromas, ganglioneuromas, Pacinian-corpuscle tumors, and hemangiomas.[1-4] Observations have been made on the tumors that occur in the skin of the body of the duck and in the web of the foot after local applications of this carcinogen.[5] An epithelial tumor with the histologic characteristics of a squamous-cell carcinoma has occurred in the skin of chickens following the local application of methylcholanthrene.[6,7] These tumors in the chicken develop within an interval of three months after treatment. Local proliferation of lymphocytes occurs in the skin of the chicken and turkey after local applications of methylcholanthrene.[8,9]

The anatomic characteristics of the respiratory tract in the duck and the mechanism by which fluids and particulate materials are removed from the lungs were described recently.[10,11] The respiratory tract in the duck is similar in many ways to that in man; however, it differs in that some of the larger bronchi communicate directly with large extrapulmonary cavities, known as air sacs. Some of these air sacs communicate directly with the humerus, sternum, and vertebrae. It is of interest to know that the duck has no lymph nodes. The terminal lymphatics continue to unite, and ultimately the major pulmonary lymph channels empty directly into the vena cava.[12]

The trachea and larger bronchi are lined by ciliated, pseudostratified epithelium with many mucin-secreting cells. The primary bronchus to each lung divides into secondary and tertiary bronchi. The latter are continuous with the air capillaries, which correspond to the alveoli in man. The smaller bronchi frequently terminate in large spaces, lined by a single layer of cuboidal epithelium.[10]

When fluid is put into the trachea of the duck, it rapidly diffuses through the wall of the smaller bronchi and air sacs to enter the lymphatics and capillaries.[11] Particulate material, such as particles of carbon in India ink, and globules of lipid material, as in liquid petrolatum, passes between the epithelial cells that line the respiratory tract and enters the adjacent stroma.[11] Macrophages may either phagocytose these particles or enter the lymphatics and capillaries directly by passing between the endothelial cells that line these channels.

To continue our studies of the effect of methylcholanthrene on the white Pekin duck, observations have been made on the pathologic changes occurring in the respiratory tract following the intratracheal injection of methylcholanthrene. The results of this experiment are reported.

Methods and Material

One hundred thirty-four white Pekin ducks were given methylcholanthrene, and ninety-nine were used as controls. The latter group of birds was used as controls in another experiment.* Twenty-

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* Effect of tobacco condensate on the respiratory tract of white Pekin ducks.

six additional ducks were given intratracheal injections of liquid petrolatum as a second control. These ducks also were used as controls in another experiment.* The ducks usually were 2 to 4 weeks of age when the experiment started. They were kept either in small groups in batteries in the animal room or in a large outside pen with other ducks. Water and food were available at all times.

The methylcholanthrene was given by one of the three following routes: (1) as crystals placed directly into the trachea, (2) as crystals suspended in liquid petrolatum, and (3) as crystals suspended in distilled water with polysorbate 80 U.S.P. (Tween 80). Treatments usually were given daily, five times each week, except for holidays. Data on the number of ducks used, the number of injections, and the time when killed are given in the various experiments. The tracheas of all the ducks were removed and examined grossly. One transverse section each was taken for histologic study from the proximal, middle, and distal thirds of the trachea of a majority of the ducks. Additional sections were removed from those tracheas that showed acute inflammation. Pathologic studies were also made of the lungs; several sections were removed for histologic study from most of the birds. The specimens were fixed in a 4% solution of formaldehyde, and paraffin sections were prepared. They were stained routinely with hematoxylin and eosin. Select sections were stained by the periodic acid-Schiff technique for mucin and the osmic-acid stain for fat.

The pulmonary tissues from 87 ducks were examined macroscopically for fluorescence, using an ultraviolet lamp (Aloe: No. 52140 ultraviolet, Mineralight, high-intensity, long-wave, 3,660 A.). This examination was made on either fresh or formalin-fixed tissues.

Experiment 1.—Approximately 2 to 3 mg. of methylcholanthrene crystals was put into the end of a 5 ml. pipette. This was put into the external larynx and then into the trachea for a distance of 2 to 3 cm. The methylcholanthrene crystals were blown, with the aid of a large rubber bulb, into the trachea of four ducks once daily for 30 days. Approximately 10 mg. of methylcholanthrene crystals was insufflated in a similar manner into the trachea of 11 ducks for the same period of time. Three of these ducks were killed on the 48th day; one, on the 90th day, and 11, between the 363d and the 369th day following the first injection.

Experiment 2.—Methylcholanthrene crystals (7.5 to 30 mg.) were put into 1 ml. of liquid petrolatum, and 0.5 ml. of this mixture was put into the trachea of 97 ducks. This was accomplished by using a small catheter, 8.0 cm. long, attached to a small syringe. The mouth of the duck was manually opened; and when the external larynx was spontaneously opened, the catheter was inserted into the trachea for a distance of 2-6 cm. In this experiment, 2 ducks were given 2 intratracheal injections; 2 were given 8; 1 was given 9; 49 were given 10; 2 were given 12; 2 were given 13; 22 were given 15; 12 were given 17; 1 was given 22, and 4 were given 26 injections. It was necessary to discontinue the intratracheal injections in some ducks for a few days because the birds developed an acute tracheitis with respiratory embarrassment. Of the ducks given the methylcholanthrene-liquid petrolatum mixture, 30 either died or were killed before the 30th day; 21, between the 31st and 99th day; 15, between the 100 and the 199th day; 19, between the 200th and the 400th day, and 12, between the 401st and the 468th day, following the time of the first injection.

One-half milliliter of liquid petrolatum was put into the trachea of 26 ducks in the same manner as was the methylcholanthrene and liquid petrolatum. Seven of these ducks were given a total of 7 to 12 injections of 0.5 ml. each and were killed immediately after the last injection; seven were given from 7 to 12 injections and were killed 24 hours after the last injection; three were given 130 injections within a period of 190 days and were killed 24 hours after the last, and five were given 15 injections and were killed 266 days after the last.

Experiment 3.—Twenty-five milliliters of a suspension of methylcholanthrene crystals (10 mg. methylcholanthrene per milliliter in a 1% solution of polysorbate 80) was given intratracheally to 22 ducks. One was killed 48 hours later; one, after 56 days; five, between the 117th and the 200th day; seven, between the 201st and the 299th day; six, between the 300th and the 315th day, and two on the 383d day.

Experimental Results

Acute and chronic inflammation of the trachea was found frequently in the 99 untreated ducks. Three of this group had focal areas of metaplasia in the trachea. Acute and chronic inflammation was present in the trachea of a majority of the ducks given the liquid petrolatum. Three of these birds also showed metaplasia; one of these was given 12 injections and was killed immediately after the last; one was given 8 injections and was killed 24 hours later, and one was given 15 injections and was killed 266 days later.

EXPERIMENT 1.—Of the 15 ducks that had methylcholanthrene crystals put into the trachea 30 times, 3 were killed 48 days after the first treatment, 1 after 90 days,

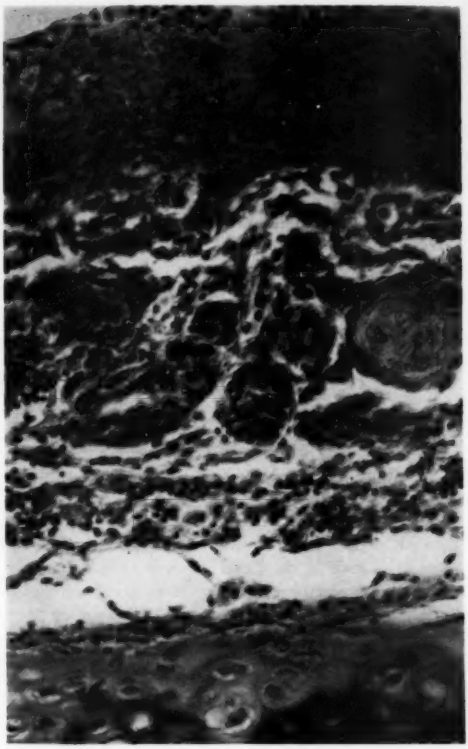


Fig. 1 (Duck 1048).—Metaplasia has occurred in the pseudostratified ciliated epithelium that normally lines the trachea of the duck. Squamous cells have infiltrated the underlying stroma. Ten milligrams of methycholanthrene crystals was put into the trachea 30 times within a period of 44 days. The duck was killed three days after the last treatment. Hematoxylin-eosin stain; × 360.

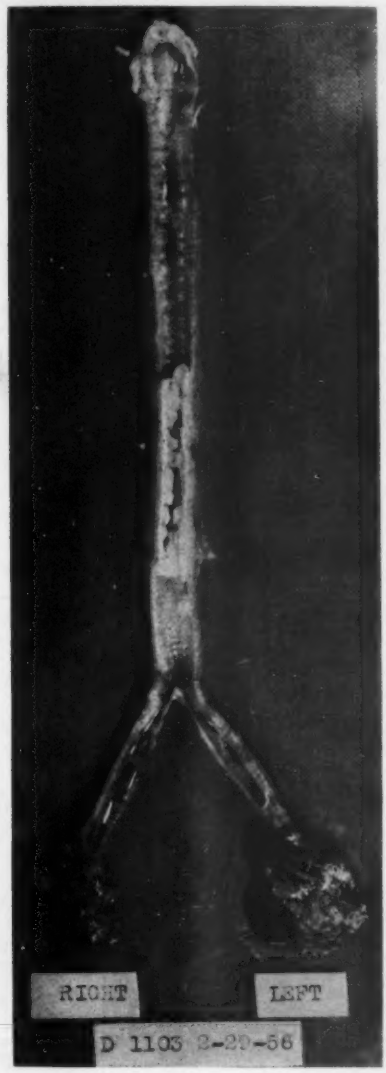
and 11 between the 363d and the 369th day. Acute and chronic inflammation was present in the proximal portion of the trachea of the four birds killed after the shorter intervals. Squamous metaplasia was conspicuous in three ducks, and in two of these there was an infiltration of the underlying stroma with squamous-like epithelial cells (Fig. 1). A chronic inflammatory reaction was present in the trachea of each of the 11 ducks observed for a year; in 3 of these there was squamous metaplasia, and in 1 some infiltration of the submucosa with squamous cells.

EXPERIMENT 2.—A majority of the ducks injected intratracheally with methylcholanthrene and liquid petrolatum showed either an acute or a chronic tracheitis, and many developed respiratory difficulty. The exudate usually was composed of necrotic, fibrinous material, which flaked off easily. It was located most frequently in the lower half of the trachea. The typical gross lesion is shown in Figure 2. This duck received 13 intratracheal injections. Respiratory difficulty was present on the 19th day, at which time this bird was killed. The trachea was 12.5 cm. in length. The exudate was located 7 cm. from the external larynx and involved an area 4 cm. in length. There was 2.5 cm. of uninvolved trachea between the lower margin of this obstruction and the bifurcation.

Twelve birds given methylcholanthrene and liquid petrolatum either died or were killed between the 16th and the 50th experimental day because of tracheal obstruction. In several of these birds the obstruction resulted from a proliferation of fibrous tissue within the lumen of the trachea (Fig. 3). Twenty-two of the ducks had extensive squamous metaplasia (Fig. 4*B*), and, of these, eight had some infiltration of the wall by squamous epithelial cells.

Metaplasia occurred within the trachea as early as the 16th day after the first of 13 intratracheal injections of the methylcholanthrene and liquid petrolatum. One duck showed metaplasia on the 29th day after receiving eight intratracheal injections of the carcinogen. Metaplasia and an infiltration of the underlying stroma with squamous cells were observed more frequently in the ducks that either died or were killed before the 55th day than in the birds that were examined after 300 days. Squamous-cell infiltration of the wall of the trachea was observed in one duck on the 23d day after the first of 17 treatments. Another duck had 13 treatments and showed a similar infiltration on the 18th day. A squamous-cell papilloma was present in the lumen of the trachea of one duck on the 36th day after the first of 15 treatments (Fig. 5).

Fig. 2 (Duck 1103).—The lumen of the trachea is occluded by an acute fibrinous exudate. Thirteen daily intratracheal injections of methylcholanthrene were given. Respiratory difficulty was present on the 19th day, at which time this bird was killed.



Nine ducks treated with methylcholanthrene and liquid petrolatum showed a proliferation of the fibrous tissue within the wall of the trachea, in addition to the metaplastic changes in the epithelium. This proliferation of fibrous tissue was localized in the trachea of six of the ducks, and in three the lumen of the trachea was markedly decreased. Metaplasia of the squamous epithelium frequently was associated with the proliferation of fibrous tissue.

Forty-nine ducks were given 0.5 ml. (7.5 mg/ml.) of the methylcholanthrene in liquid petrolatum four times. This dose was then increased to 15 mg/ml. for six more times. These 10 injections were given within a period of 15 days. The treatments were discontinued in this group of ducks because some of the birds were showing clinical evidence of tracheal obstruction. One duck died 24 hours after the 9th injection, and 11 either died or were killed because of a tracheal obstruction during the 10 days that followed the 10th injection of the carcinogen. In each bird there was an extension exudate that fluoresced under ultraviolet light. Thirteen additional ducks either died or were killed because of tracheal obstruction within the first month following the last intratracheal injection of this carcinogen. The obstruction resulted from an acute tracheitis accompanied by an extensive fibrinous exudate, much as is shown in Figure 2. After one month some

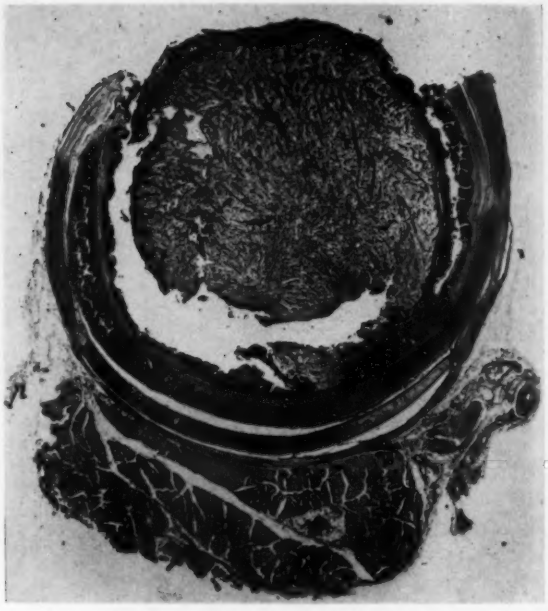
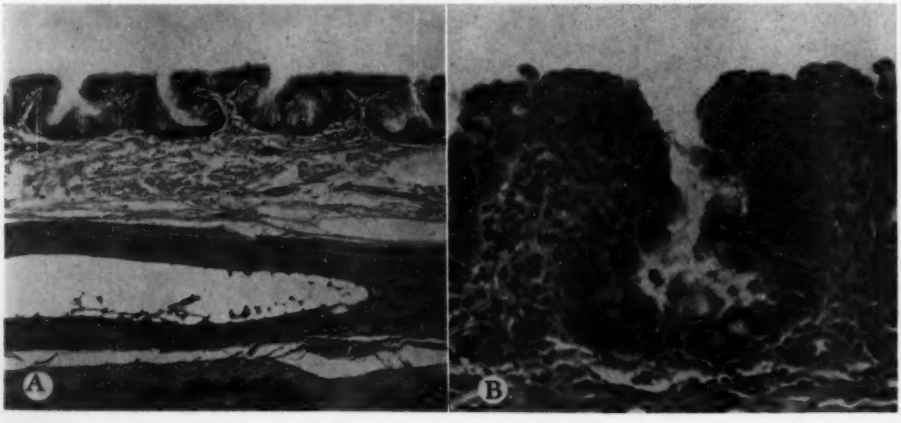


Fig. 3 (Duck 1208).—The trachea is occluded by a local proliferation of fibrous tissue. This bird was given 15 intratracheal injections within a period of 18 days and was killed because of respiratory difficulty on the 23d day after the first injection.

of the ducks were beginning to show tracheal obstruction in which fibrous tissue had replaced the acute exudate (Fig. 6). Squamous metaplasia and lymphocytic infiltration of the submucosa of the trachea were associated with this proliferation of fibrous tissue.

Within the first 34 days following the last intratracheal injection of methylcholanthrene, 32 ducks in the latter group had died, and, of these, 29 died or were killed because of tracheal obstruction. The ducks continued to die as the result of fibrous occlusion of the trachea. On the 248th day

Fig. 4.—(*A*) Normally the trachea is lined by pseudostratified ciliated epithelium. (*B*) Duck 1100. Metaplasia frequently occurs in the epithelium that lines the trachea after the intratracheal injection of methylcholanthrene. This bird was given 17 intratracheal injections of methylcholanthrene in liquid petrolatum within a period of 27 days and died 24 hours after the last injection. Hematoxylin-eosin stain; × 380.



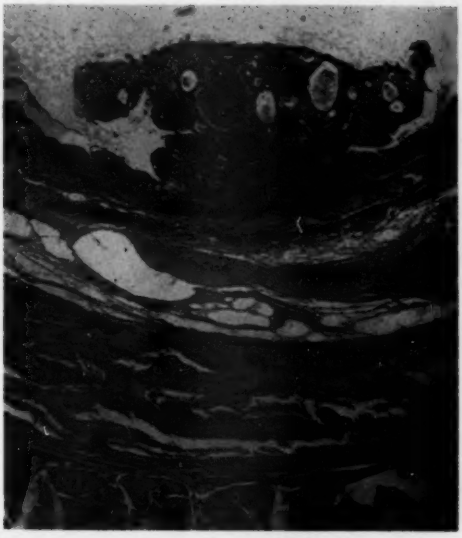
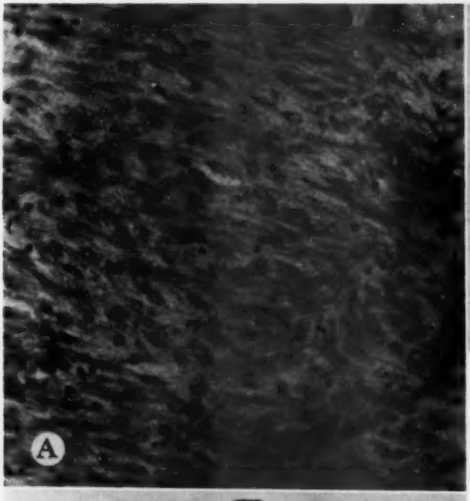
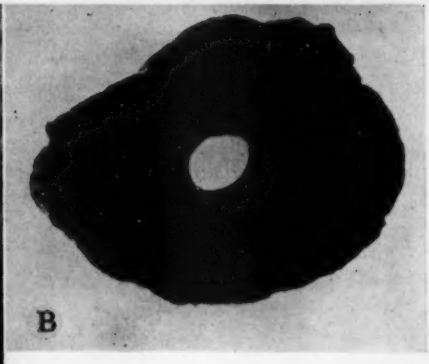


Fig. 5 (Duck 1197).—A small papilloma projects into the lumen of the trachea. This duck was given 15 intratracheal injections of methylcholanthrene in liquid petrolatum within a period of 18 days and was killed on the 36th day after the first treatment. Hematoxylin-eosin stain; × 48.





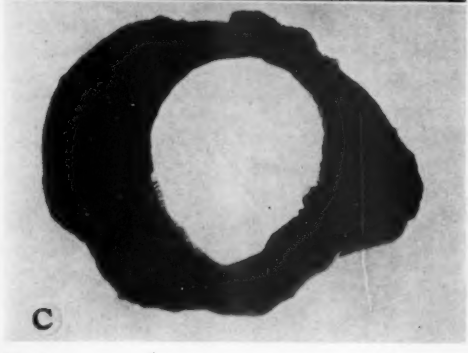


Fig. 6.—(*A*) Duck 1193. Beneath the metaplastic change in the trachea is a marked proliferation of fibrous tissue. This duck was given 15 intratracheal injections of methylcholanthrene in liquid petrolatum within a period of 18 days and died on the 37th day after the first injection. Hematoxylin-eosin stain; reduced to 85% of mag. × 400.

(*B*) Duck 1482. The lumen of the trachea is markedly decreased as a result of the proliferation of fibrous tissue. Photograph of a transverse section of the trachea.

(*C*) Lumen of a normal trachea. Photographed in the same way as *B*.

Fig. 7.—(*A*) Duck 1207. The lumen is markedly decreased by the fibrinous exudate. Photographed with routine artificial lights.

(*B*) This exudate fluoresces under ultraviolet light, suggesting the presence of methylcholanthrene. This duck was given 13 intratracheal injections of methylcholanthrene in liquid petrolatum and was killed approximately four hours after the last. Photographed with ultraviolet light.

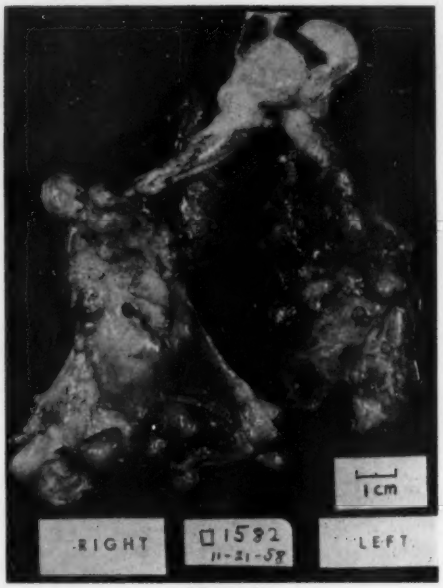


Figure 8

Fig. 8.—Twenty-five milliliters of a 1% solution of polysorbate 80 containing 250 mg. of methylcholanthrene crystals was given intratracheally to an adult duck, and he was killed 225 days later. Tumors histologically characteristic of carcinomas and sarcomas were present in the lung and in the wall of the abdominal air sac.

following the last intratracheal injection, Duck 1475 was killed because of respiratory difficulty resulting from a fibrous occlusion of the trachea similar to that shown in Figure 6. On the 291st day Duck 1496 was killed because of a similar obstruction.

Only 14 ducks from the second group of 49 birds given liquid petrolatum and methylcholanthrene were alive at the end of one year. There was no gross evidence of tumor in the respiratory tract of any of these ducks. One bird died on the 377th day; one was killed on the 389th and another on the 430th day, and 11 were killed on the 468th experimental day. There were some firm, yellow masses 0.2 to 1.0 cm. in diameter, surrounded by scar tissue, in the lesser abdominal air sac of one duck killed on

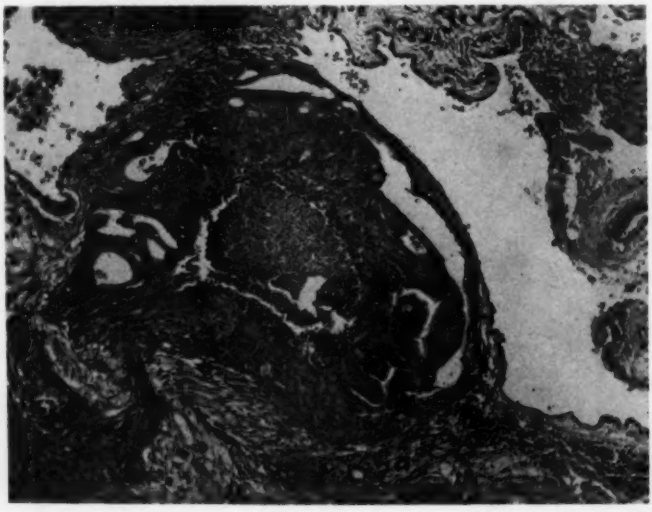


Fig. 9.—This section from the specimen shown in Figure 8 was removed from the lung tissue adjacent to the wall of the bronchus communicating with the abdominal air sac. The epithelial cells are surrounded by a zone of spindle-shaped cells. The former are characteristic of neoplasia. Hematoxylin-eosin stain; reduced to 92% of mag. × 110.

the 468th day. This yellow material fluoresced under ultraviolet light. A bird killed on the 430th day and another on the 468th day had typical cirrhotic livers.

Since methylcholanthrene fluoresces in the presence of ultraviolet light, the tracheas from 26 normal ducks were observed under ultraviolet light. There was no fluorescence. Neither did the tracheas of the 16 ducks given intratracheal injections of liquid petrolatum fluoresce. The tracheas of 45 ducks given liquid petrolatum and methylcholanthrene were examined for fluorescence. A tracheal exudate was present in 13 of these ducks, and all but one trachea fluoresced (Fig. 7). Fluorescence was absent usually when scarring was present in the trachea. Ten ducks were given one intratracheal injection of methylcholanthrene in liquid petrolatum, and five were killed within 5 to 15 minutes. There was no exudate in the trachea; however, the trachea and the lung did fluoresce. The other five ducks in this group were killed 48 hours after the methylcholanthrene was put into the trachea. There was no macroscopic evidence of inflammation in the trachea; however, fluorescence did occur in the trachea of three of the five ducks and in the lung of one. Six ducks similarly treated with methylcholanthrene and liquid petrolatum were killed 72 hours later. An exudate which fluoresced under ultraviolet light was present in the trachea of one bird. There was no exudate or fluorescence of the trachea in the other five ducks. The lung fluoresced in each of the six ducks given one injection of methylcholanthrene in liquid petrolatum and killed after 72 hours. Five ducks were similarly treated and killed 120 hours later; no exudate was present in these ducks, and no fluorescence was present in the trachea or the lungs.

This experiment on fluorescence of the respiratory tract in ducks was repeated. Methylcholanthrene in liquid petrolatum was given intratracheally to 17 ducks daily for nine days. Twelve ducks thus treated either died or were killed within 72 to 288 hours. An extensive tracheal exudate, which fluoresced, was present in each of these birds. Three of the ducks died after 312 hours; there was a thick exudate present, and it fluoresced. Another duck died after 384 hours, and an exudate was present in the trachea, but it did not fluoresce. The last duck in this experiment died after 408 hours. An extensive tracheal exudate was present, but it was questionable as to whether or not there was any fluorescence within the exudate.

From these observations on methylcholanthrene and fluorescence, it would appear that this carcinogen disappears from the trachea and lungs, within an interval of 120 hours or less, after an intratracheal injection when there is no macroscopic inflammation. Fluorescence may persist in the trachea, however, for 312 hours if an exudate is present.

To demonstrate macroscopically the persistence of methylcholanthrene in an area of inflammation, 0.2 ml. of acetone was injected directly through the external larynx into the trachea of 10 ducks. Within 20 hours a severe local inflammatory reaction was present and persisted at least 216 hours. Three ducks were given a similar intratracheal injection of acetone, and 24 hours later an intratracheal injection of 0.5 ml. of methylcholanthrene in liquid petrolatum. Two of the ducks died within 24 hours with a severe tracheitis, and the third bird died 120 hours later; the exudate in the trachea of all three fluoresced.

Six ducks were given 0.5 ml. of acetone intratracheally, and the dose was repeated every 48 hours for three times. Twenty-four hours following the last injection of acetone 0.5 ml. of methylcholanthrene in liquid petrolatum was injected intratracheally. Sixty-five hours later the six ducks were killed. An exudate that fluoresced was present in the trachea of each of these six birds. This experiment was repeated using nine ducks; only one intratracheal injection of acetone was made. Five of the ducks were killed 120 hours after the methylcholanthrene in liquid petrolatum was given. An exudate was present in the tra-

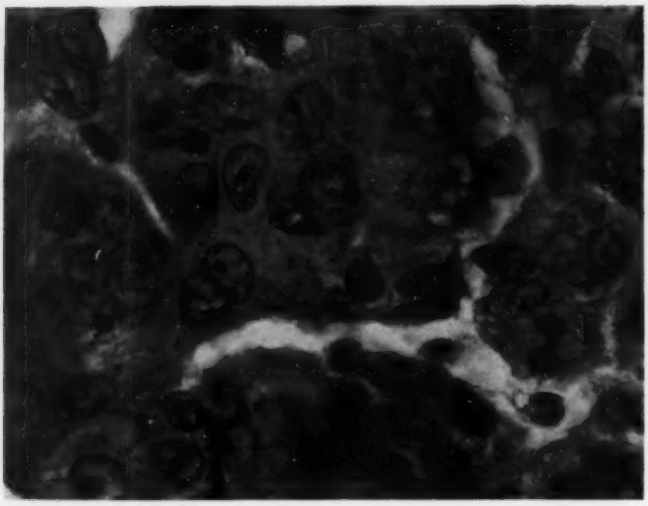


Fig. 10.—Epithelial cells from the tumor shown in Figure 9. Hematoxylin-eosin stain; reduced to 62% of mag. × 1,170.

chea that fluoresced under ultraviolet light. Four additional ducks were killed 216 hours after the intratracheal injection of methylcholanthrene and liquid petrolatum. An exudate was present in the trachea that fluoresced with ultraviolet light.

Fig. 11.—Some of the spindle-shaped cells occurring in the tumor shown in Figure 9. It is difficult to be sure whether or not these cells are mesodermal or ectodermal in origin. Hematoxylin-eosin stain; × 1,170.

EXPERIMENT 3.—In the preceding experiments the methylcholanthrene produced the most conspicuous lesion in the trachea. Preliminary experiments had shown that 200 ml. of water could be put directly into the respiratory tract of an adult duck within a period of two hours without significant clinical manifestations occurring.[11] Twenty-one ducks given an intratracheal injection of methylcholanthrene in polysorbate 80 survived from 56 to 384 days, and 15 of them developed neoplasms within the respiratory tract and in the abdominal air sacs. Since this was the first time in which methylcholanthrene suspended in polysorbate 80 had been given to ducks, the experiment is being repeated. A group of birds is being given only polysorbate 80 as a control. Pathologic studies have shown carcinomas and sarcomas in both lung and wall of the air sacs (Figs. 8, 9, 10, 11). Detailed discussion of the types of neoplasms and the time of their appearance will be reported subsequently in a much larger group of ducks given varying amounts of methylcholanthrene.

Comment

A single intratracheal injection of methylcholanthrene crystals suspended in distilled water to which had been added a 1.0% solution of polysorbate 80 produced neoplasms within the lung and abdominal air sacs of white Pekin ducks. Carcinomas and sarcomas occurred. Although in this preliminary study no control observations have been made using only polysorbate 80, it seems most likely that the carcinogenic effect has resulted from the methylcholanthrene. Other experiments are now in progress in which two different quantities of methylcholanthrene in polysorbate 80 have been given to a large number of ducks. A similar volume of polysorbate 80 has been given as a control. A report of the results of these studies will be made later, including a pathologic study of the neoplasms.

Methylcholanthrene suspended in liquid petrolatum produces extensive inflammation and necrosis of the mucosa of the trachea. Metaplasia frequently occurs; associated with this metaplasia in some of the ducks is an infiltration of the submucosa with squamous cells that might be interpreted as neoplasia. A similar change occurred in some of the birds given only the methylcholanthrene crystals. Two of the four ducks in the latter group killed within 58 days after the first treatment showed metaplasia, with some infiltration of the adjacent stroma. Three ducks similarly treated and killed a year later showed metaplasia, and one of these showed some infiltration of the submucosa. Obviously, metaplasia occurs and persists for long periods without any histologic evidence to suggest an associated neoplasia. In the duck it becomes most difficult, and often impossible, to differentiate these two processes. This same problem arises with reference to the inflammatory and neoplastic changes in the lung and air sacs.

It is impossible to know the quantity of methylcholanthrene that reaches the lungs when this carcinogen is suspended in liquid petrolatum. It may be that in these experiments the amount of the carcinogen that reached the pulmonary tissues and air sacs was not sufficient to produce neoplasms. No one, of course, knows how much methylcholanthrene is necessary to produce a tumor in the respiratory tract of the duck. It is obvious from the fluorescence studied that the methylcholanthrene put into the trachea remains in the respiratory tract for a period of time and is not immediately removed through the action of the cilia. Furthermore, methylcholanthrene persists for a longer period in the presence of an inflammatory exudate.

A high percentage of the ducks given the methylcholanthrene in polysorbate 80 showed an amyloid-like substance in the liver, spleen, pancreas, adrenal, and kidney. The hepatic lesion is the same as that previously reported in ducks where methylcholanthrene in acetone was applied locally to the skin.[13] Similar amyloid-like material has been observed in the liver of white

Pekin ducks given tobacco condensate intratracheally.

Summary

Methylcholanthrene suspended in liquid petrolatum when put into the trachea of white Pekin ducks usually produces an acute tracheitis. The exudate frequently is so extensive that death results from the obstruction. Squamous metaplasia occurs in the trachea of birds that developed a less severe inflammation reaction. Ducks may die of respiratory obstruction at a later time, as the result of either local proliferation of fibrous tissue or a papilloma that develops within the lumen of the trachea.

Metaplasia in the trachea is a common finding in these ducks. It is more frequent in those birds given methylcholanthrene and liquid petrolatum; however, it does occur in the trachea of a few of the normal ducks and in those receiving only liquid petrolatum. In some of the tracheas showing metaplasia there is also an infiltration of squamous cells into the underlying stroma. This process is suggestive of neoplasia, but no macroscopic squamous-cell carcinoma has been observed in the trachea of any of these ducks.

Methylcholanthrene suspended in water with polysorbate 80, when given intratracheally, produces carcinomas and sarcomas in the lung and in the air sacs of white Pekin ducks.

Department of Pathology, University of Texas Medical Branch.

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News and Comment

ANNOUNCEMENTS

Seventh Conference International Society of Geographical Pathology, London, June 29-July 1, 1960.—The chief topic of the conference is Eclampsia and Preeclampsia. Members of the Society or other persons wishing to have papers placed on the program should send the title of their paper and a 250-word abstract to Prof. Fred C. Roulet, General Secretary, International Society of Geographical Pathology, Schäublin-Strasse 17, Basle 24, Switzerland, in time to reach him by Dec. 30, 1959. Papers concerned with the chief topic of the conference or papers of general geographical-pathological interest are suitable.

National Cancer Institute Directory.—The National Cancer Institute has just published the first directory of its research fellows since the fellowship program was established in 1937. Section I of the Directory contains the names of 924 persons whose fellowships had been completed previous to April 1, 1958; Section II contains the names of 245 persons whose fellowships extended beyond that date. A brief statement about each fellow tells the highest degree he held at the time of his appointment; the name of the school granting the degree; the type, duration, and place of his fellowship, and the name of his research project. The present position of those whose fellowships had terminated by April 1, 1958, is also given. The introduction to the Directory discusses the history of the program, which was the first of its kind to be supported by the Federal Government.

Most of the former fellows are now engaged in research or teaching or both. About half of the fellows appointed in the early years of the Institute are now permanent members of the Institute's staff in key research positions, and a number of the later fellows have also joined the staff.

Copies of the Directory have been sent to the libraries of appropriate institutions, including four-year colleges, medical schools, and foundations supporting research. A limited number of free copies are available from the National Cancer Institute, Attn.: Information Officer. The 134-page booklet, "Research Fellows of the National Cancer Institute, 1938-1958," is on sale by the Superintendent of Documents, Government Printing Office, Washington 25, D.C., for 45 cents a copy.

PERSONAL

Appointment of Dr. George Margolis.—Dr. George Margolis has been made professor and chairman of the Department of Pathology at the Medical College of Virginia, in Richmond.

Appointment of Dr. Orville T. Bailey.—Dr. Orville T. Bailey has been appointed professor of neuropathology at the University of Illinois College of Medicine. He succeeds Dr. Percival Bailey, who retired from the University of Illinois on Sept. 1, 1959. Dr. Orville Bailey is president-elect of the American Association of Neurolopathologists.

DEATHS

Dr. Arthur H. Sanford Dies.—Dr. Arthur H. Sanford, Rochester, Minn., died on April 28, 1959, at the age of 77.

Books

Perspectives in Virology. Edited by Morris Pollard. Price, $7. Pp. 312. John Wiley & Sons, Inc., 440 Fourth Ave., New York 16, 1959.

This volume is a collection of fifteen papers presented at a symposium held in 1958, and participated in by 117 leading American and European virologists. A foreward by Dr. Selman Waksman pays tribute to the late Dr. F. R. Beaudette, in whose memory this symposium was held. Fifteen papers review the present knowledge and future research potentialities in virology. Subjects covered include the chemical nature of viruses, viral multiplication, genetic interaction between bacteriophage and bacteria, in vitro culture methods, and aspects of viral neoplasia. Also discussed are specific viral diseases, among them measles, hog cholera, Asian influenza, and the recently recognized viral diseases in man caused by entero- and respiratory-tract viruses. The extemporaneous discussion of these papers is included. A delightful epilogue, "Tulipomania and the Benevolent Virus," by Dr. René J. Dubos, considers viral infections from a biological (and philosophical) point of view.

Lungenkarzinom und Lungenadenom. Second Edition. By Prof. Dr. J. Baló. Price, not stated. Pp. 379, with 203 illustrations. Verlag der Ungarischen Akademie der Wissenschaften, Budapest, Hungary, 1959.

The over-all importance of lung cancer in today's medicine calls for monographs such as this. We read in the introduction that this volume is actually the second edition, published ten months after the first edition was released! Basically an account of the author's experience with 200 cases of pulmonary primary carcinoma and 23 cases of bronchial adenomata (surgical and autopsy material), it includes such topics as etiology of lung cancer, carcinoma in situ, precancerous changes, and biopsy and exfoliative cytology.

An introductory bibliography of the "most important works and monographs on lung cancer" cites several Russian monographs and articles unknown to most American pathologists. The chapter on pathological anatomy of lung cancer is rather conventional, reviewing the various gross and microscopic classifications. From the statistical data given, no essential differences are apparent between lung cancer in Hungary and that in the United States. The chapter on histogenesis suffers from little critical appraisal. Alveolar-cell carcinoma is interpreted as neoplasia of the epithelial cells lining the alveoli. Unfortunately, more recent anatomical work on this controversial question is omitted from the discussion. Possible etiological mechanisms are treated extensively. Questions such as tuberculosis, bronchiectasis, and pulmonary infarcts and their relation to lung cancer are discussed at length.

The second part of the book is devoted to bronchial adenomata. A special chapter on experimental adenomata in mice records the author's experience in this field.

The book is written in clear German. Macro- and microphotographs are beautifully reproduced, though magnifications are not given. References are complete. We regret the lack of clinical-pathological correlation in an otherwise thorough and carefully prepared monograph.

A Textbook of Medicine. Tenth Edition. Edited by Russell L. Cecil, M.D., Sc.D., and Robert F. Loeb, Sc.D., D. Hon. Causa, LL.D., and others. Price, $16.50. Pp. 1,665, with 182 figures. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1959.

The tenth edition of "A Textbook of Medicine" follows in the grand tradition of the earlier publications. One can readily appreciate why this text is considered one of the "bibles of internal medicine" by third- and fourth-year medical students. And, although written primarily for medical students, it will serve the internist well.

The major additions to the tenth edition include advances of the past four years in knowledge of diseases, such as ECHO viral infection, cytomegalic inclusion disease, acute pseudomembranous enterocolitis, hepatic coma, pantothenic acid deficiency, carcinoidosis, and many other entities. Altogether, thirty-six new diseases or syndromes are discussed in detail. The short introductory chapter on steroid physiology and metabolism deserves special mention.

One criticism can be made, but only in light of the tremendous scope of the text and the necessary limitations of space. Nevertheless, such a text should have a chapter on

dermatology, with pictures and discussion of the common diseases seen in the practice of internal medicine. The editors might argue that there are already good dermatological texts available, but other systemic diseases are covered by their own volumes, also.

From the viewpoint of the pathologist, this edition is highly recommended as a general medical reference. The pathologic physiology of various diseases is adequately covered, but the pathology in some instances is lacking.

An index of 89 pages adds immeasurably to the value of the text.

Atlas of Tumor Pathology, Section XII, Fascicle 40: Transplantable and Transmissible Tumors of Animals. By Harold L. Stewart, M.D.; Katharine C. Snell, M.D.; Lucia J. Dunham, M.D., and Samuel M. Schlyen, M.D. Price, $3.50. Pp. 378, with 287 illustrations. Armed Forces Institute of Pathology, Washington 25, D.C., 1959.

The largest fascicle in this series so far is a welcome addition to the "Atlas of Tumor Pathology." In the introduction, a historical review is presented, and sources of transplantable tumors, techniques, and results of transplantation are described. In the following chapters some 50 transplantable and transmissible tumors of animals are classified according to the site of origin, such as skin, melanin-forming tissue, subcutaneous tissues, muscle, bone, hematopoietic tissues, lung, alimentary tract, kidney, reproductive organs, mammary gland, endocrine glands, blood vascular system, neural tissue, and undetermined sites. Supplementary classifications according to animals (mouse, rat, rabbit, dog, chicken, hamster, guinea pig, and frog) are given. Each individual chapter is divided into a definition of the tumor, history and description of the original tumor, transplantation or transmission studies, and a description of the current tumor. Excellent macroscopic and microscopic illustrations accompany this most valuable guide. No doubt, this fascicle will establish its place as the most useful "handbook" to the experimental oncologist.

Die Pathologie des kindlichen Pankreas. By Dozent Dr. med. Gerhard Seifert. Price, 52 DM. Pp. 151, with 109 illustrations. VEB Georg Thieme, Hainstr. 17/19 Aufg. C, Leipzig C. 1, 1956.

This monograph presents the pathology of the pancreas of prematures, infants, children, and adolescents. The basis of this study is over 500 autopsies with careful dissection and microscopic examination of the pancreas. In an introductory chapter, normal embryology, morphology, and physiology are concisely described. Disorders of secretion (*Dyschylie*) are discussed extensively, as is the most important disease of the pancreas at this age, namely, fibrocystic disease. One chapter is dedicated to the pancreatic alterations found in various disease states. These changes are well documented by photomicrographs of high quality. The bibliography is exhaustive. The monograph is warmly recommended.

Pathology. Second Edition. By Peter A. Herbut, M.D. Price, $18.50. Pp. 1,516, with 1,506 illustrations. Lea & Febiger, 600 S. Washington Sq., Philadelphia 6, 1959.

This reliable and readable text has been a ready reference for students and clinicians alike, mainly because of its succinct style and well-organized format. A well-illustrated basic presentation of special pathology, it has emphasized pathologic anatomy and convenient subdivisions. It now appears in its second edition.

In the new edition, the portion devoted to general pathologic processes has been increased to about 260 pages of the 1,516-page volume. The additional material will be welcomed by students and teachers, since much of it has helped to shift some of the emphasis to pathogenic mechanisms and physiopathology. Included are sections on carbohydrate and lipid metabolism, hemoglobin, iron metabolism, bilirubin, vitamins, and disturbances of fluid balance, contributed by qualified collaborators.

As before, each chapter in the remainder of the book deals with an organ or part of an organ system. Pathologic processes are considered under the headings: congenital anomalies, degenerations, inflammations, physical disturbances, and tumors. Some of the previously sketchy sections have been enlarged and brought up to date. Dr. Bernard J. Alpers has again written the chapter on the central nervous system. In keeping with the attempt to expand the treatment of pathogenic mechanisms and physiopathology, each of the chapters in the new edition begins with a brief presentation of the pathologic physiology of the organ to be considered by one of 20 contributors. This usually includes a brief survey of

the normal physiology, a discussion of the deviations from normal, the morphologic changes underlying the altered physiology, and an outline of pertinent laboratory tests.

The additions and expansions have necessitated 288 additional pages and 213 additional photographs, rendering this already encyclopedic text even more imposing. However, the terse style and rigid organization keep the volume quite manageable.

The Plasma Proteins. By Paul G. Weil, M.D., Ph.D. Price, $3.50. Pp. 133. J. B. Lippincott Company, E. Washington Sq., Philadelphia 5, 1959.

The relationship of the various plasma proteins to health and disease forms the basis of this monograph. The author has generally succeeded in condensing a great number of facts concerning the plasma proteins from the clinical point of view. Most of the significant information has been included, and gaps in knowledge have been pointed out. Because of the desire to present the material as briefly as possible, occasional statements have become categorical and are open to criticism. Only those references reviewing a particular part of the subject or dealing with fundamental aspects of it have been included. This is unfortunate, since many fascinating facts are given, without indicating a source for further information.

Immunity and Virus Infection. By Victor A. Najjar, M.D. Price, $10.50. Pp. 262. John Wiley & Sons, Inc., 440 Fourth Ave., New York 16, 1959.

This volume is a collection of the papers that were presented at a symposium on Immunity and Virus Infection, held in May, 1958, at the Vanderbilt School of Medicine. Much valuable material dealing with basic research, as well as biological and clinical investigations, has been assembled in this volume. The section on immunity considers antibody formation based on clonal selection at the cellular level, the role of antigen at a molecular level, delayed hypersensitivity, immunological tolerance, the properdin system, the allergic state and "immunological diseases," some immunological methods, and the genesis of fever in infection. The section on virology includes discussions of some plant, bacterial, and animal viruses. Considerable space is devoted to a consideration of immunology and epidemiology of poliomyelitis as influenced by killed- and live-virus vaccine.

The Harvey Lectures, Series 53. By Drs. John H. Dingle, Frank Fenner, H. Fraenkel-Conrat, Joshua Lederberg, Arthur Kornberg, Albert H. Coons, Daniel Mazia, J. Gough, and John H. Gibbon Jr. Price, $7.50. Pp. 312. Academic Press, Inc., 111 Fifth Ave., New York, 1959.

The current volume of this distinguished lecture series continues the tradition of excellence. The lecture titles are as follows: An Epidemiological Study of Illness in Families, by Dr. J. H. Dingle; Myxomatosis in Australian Wild Rabbits, by Dr. Frank Fenner; Structure and Infectivity of Tobacco Mosaic Virus, by Dr. H. Fraenkel-Conrat; Bacterial Reproduction, by Dr. Joshua Lederberg; Enzymatic Synthesis of Desoxyribonucleic Acid, by Dr. Arthur Kornberg; Some Reactions of Lymphoid Tissues to Stimulation by Antigens, by Dr. A. H. Coons; Cell Division, by Dr. Daniel Mazia; Correlation of Roentgenological and Pathological Changes in Some Diseases of the Lung, by Dr. J. Gough, and Extracorporeal Maintenance of Cardiorespiratory Functions, by Dr. J. H. Gibbon Jr. It is unfortunate that delay in publication of these lectures is necessary; nearly two years has elapsed since the first lecture of the present series was presented.

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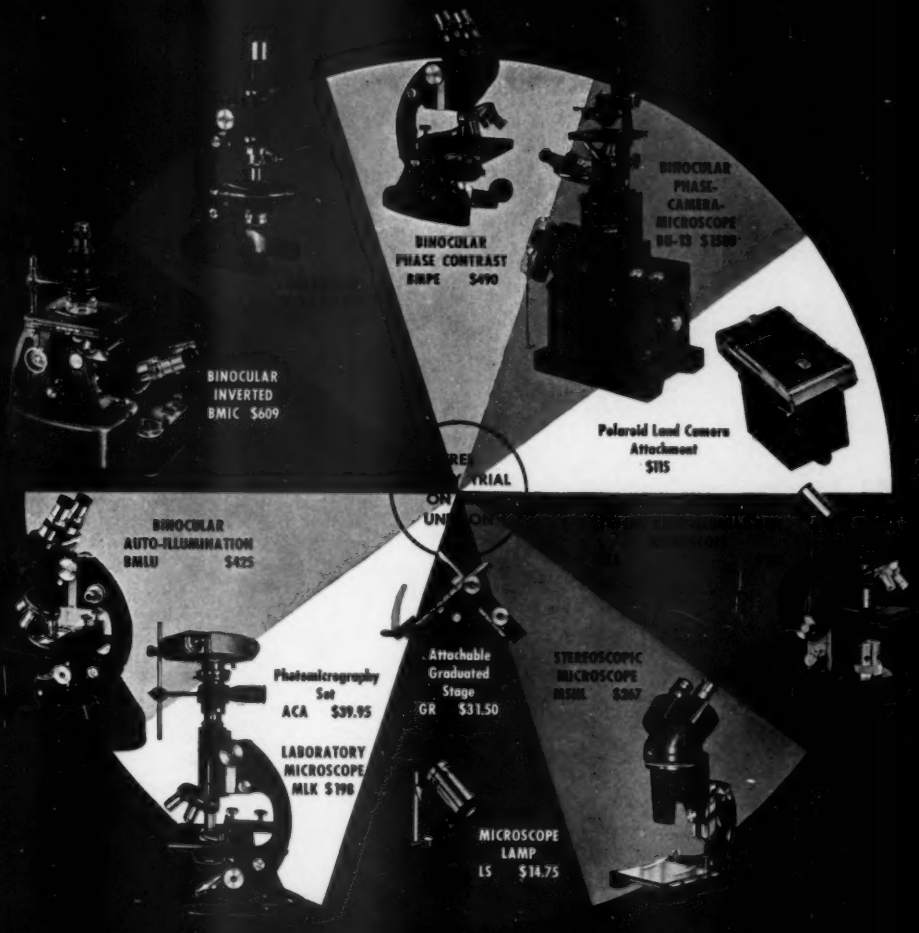

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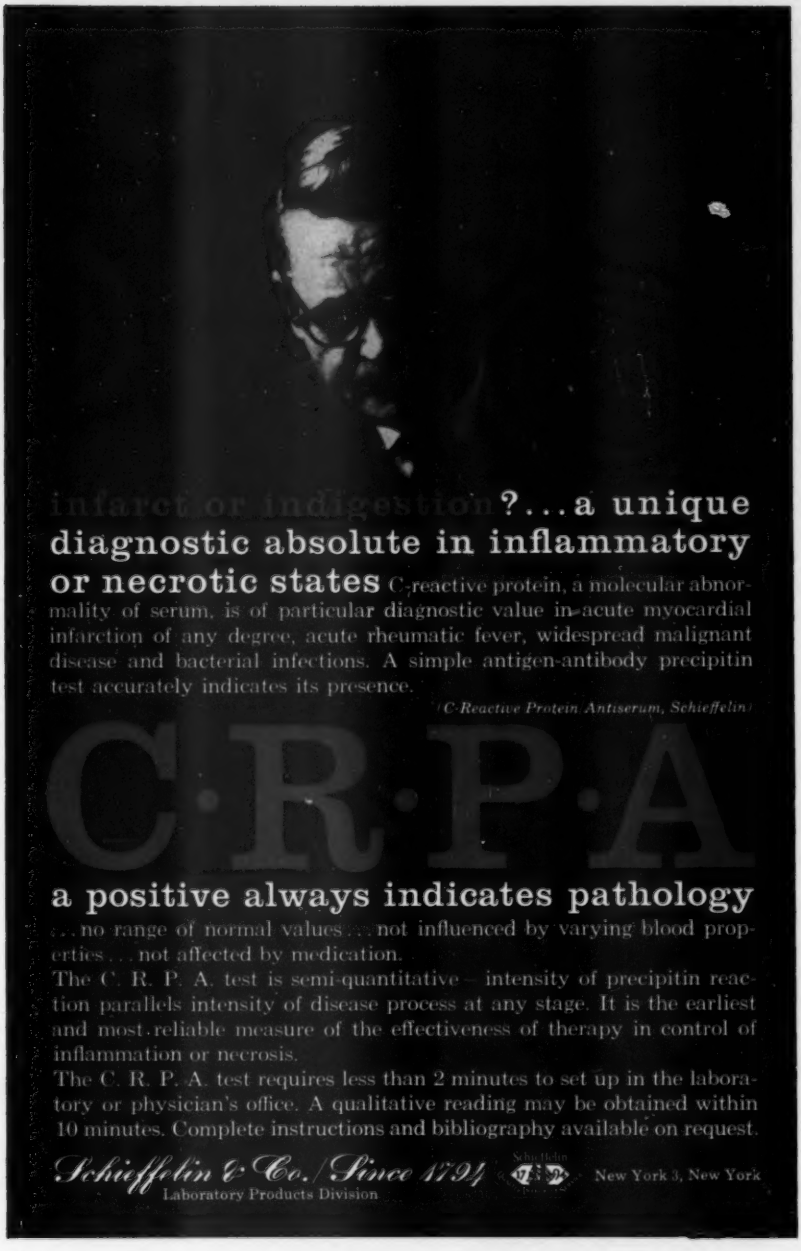
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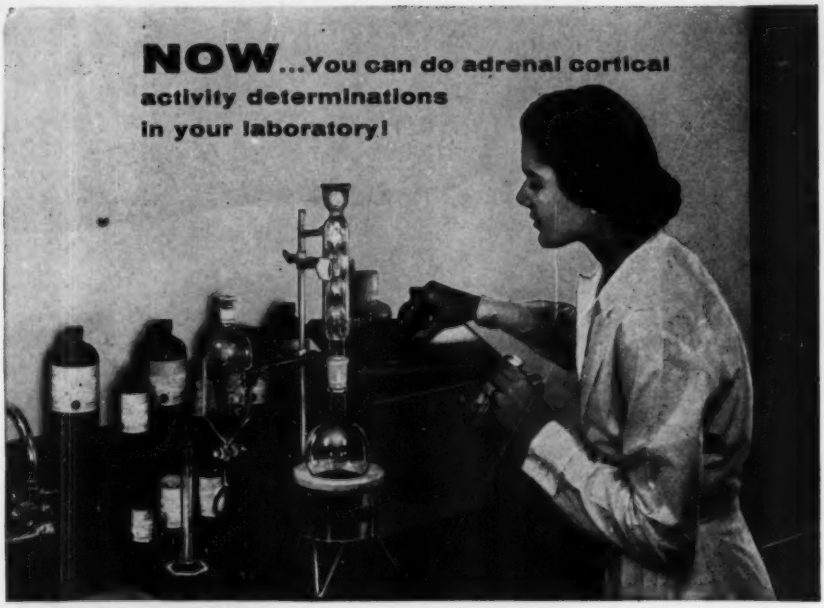

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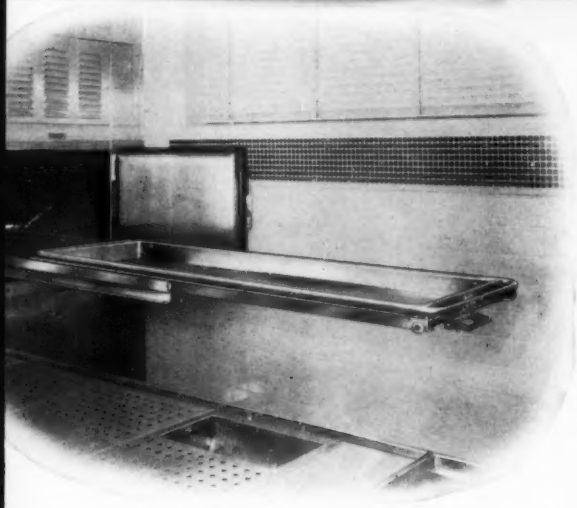

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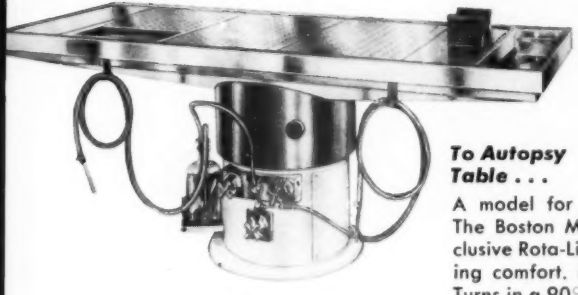

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